Mehmet Akif Ersoy Üniversitesi

Sağlık Bilimleri Enstitüsü Dergisi

SBE

SAĞLIK BİLİMLERİ ENSTİTÜSÜ

AĞUSTOS/AUGUST **2022**
CİLT/VOLUME **10**
SAYI/ISSUE **2**

Mehmet Akif Ersoy University

Journal of Health Sciences Institute

E-ISSN: 2148-2837

ISSN: 2148-2837

MEHMET AKİF ERSOY ÜNİVERSİTESİ SAĞLIK BİLİMLERİ ENSTİTÜSÜ DERGİSİ

Mehmet Akif Ersoy University Journal of Health Sciences Institute

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Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi yılda 3 sayı olarak yayımlanır (Aralık-2019 itibariyle). Dergi, *DOAJ, Google Scholar, SciLib, Researchbib, SOBIAD, Türkiye Atıf Dizini* gibi ulusal ve uluslararası indeksler tarafından taranmaktadır.

Yıl/Year: 2022 – Cilt/Volume:10 – Sayı/Issue: 2

Yayın Kurulu / Advisory Board

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I- Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi Genel Bilgiler

Mehmet Akif Ersoy Üniversitesi (MAKÜ) Sağlık Bilimleri Enstitüsü Dergisi, Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü'nün yayın organıdır. Derginin kısaltılmış adı "MAKÜ Sag. Bil. Enst. Derg" dir. Yılda 2 kez yayınlanır. MAKÜ Sağlık Bilimleri Enstitüsü Dergisi sağlık bilimleri, (veteriner, tıp, diş hekimliği, hemşirelik ve spor bilimleri) alanlarında temel ve klinik hakemli bilim yazılarının yayınlandığı hakem-denetimli bir dergidir. Derginin dili İngilizce'dir. Dergiye gönderilen yazıların başka herhangi bir dergide yayınlanmamış, yayına kabul edilmemiş ya da yayınlanmak üzere değerlendirme aşamasında olmaması gerekir. Bu kural bilimsel toplantılarda sunulan ve özeti yayınlanan bildiriler için geçerli değildir. Ancak, bu gibi durumlarda bildirinin sunulduğu toplantının adı, tarihi ve yeri bildirilmelidir. Makalelerin formatı "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (http:/www.icmje.org/)" kurallarına göre düzenlenmelidir.

Gönderilen yazılar yayın kuruluna ulaştıktan sonra öncelikle, yazım kurallarına uygunluğu yönünden değerlendirilir; sonucu yazara dört hafta içinde bildirilir. Yazının, gerek teknik özellikleri gerekse genel kapsamı açısından derginin genel yayın ilkelerine uygun bulunmaması durumunda yazı reddedilir. Ya da, gerekirse, yazar(lar)ın yazıyı yazım kurallarına uygun biçimde yeniden göndermeleri istenebilir. Yeniden gönderilen yazılar benzer bir teknik incelemenin ardından yazım kurallarına uygun ise danışman denetimi sürecine alınır. Yazı, editör ve yardımcı editörler ile yazının başlık sayfasını görmeyen en az iki danışmana gönderilerek incelenir. Yazı, yayın kurulunun belirlediği ve bilimsel içerik ve yazım kuralları açısından değerlendirilir. Editör ve yardımcı editörler gerek gördüğünde makaleyi üçüncü bir danışmana gönderebilir. Hakem belirleme yetkisi tamamen editör ve yardımcı editörler ve yayın kuruluna aittir. Danışmanlar belirlenirken derginin uluslararası yayın danışma kurulundan isimler seçilebileceği gibi yazının konusuna göre ihtiyaç duyulduğunda yurt içinden veya yurt dışından bağımsız danışmanlar da belirlenebilir. Daha sonra, danışman raporları dikkate alınarak ve gerekirse yazar(lar)la tekrar iletişim kurularak yayın kurulunca son redaksiyon yapılır. Yazıların kabulüne editör karar verir.

Editör yayın koşullarına uymayan yazıları; düzeltmek üzere yazarına geri gönderme, biçimce düzenleme veya reddetme yetkisine sahiptir. Yazılarını geri çekmek isteyen yazarlar bunu yazılı olarak editöre bildirmek durumundadır. Editör görülen lüzum halinde bazı makaleler hakkında yayın yürütme kurulunun görüşüne başvurur. Bu değerlendirme süreci dergiye gönderilen yazı türlerinden araştırma yazılarını, olgu sunumlarını ve özgün yazıları kapsar. Diğer yazı türlerindeki yazılar doğrudan yayın kurulunca değerlendirilir. Dergiye gönderilen yazılar yayınlansın ya da yayınlanmasın geri gönderilmez. Tüm yazarlar bilimsel katkı ve sorumluluklarını ve çıkar çatışması olmadığını bildiren toplu imza ile yayına katılmalıdır. Araştırmalara yapılan kısmi de olsa nakdi ya da ayni yardımların hangi kurum, kuruluş, ilaç-gereç firmalarınca yapıldığı dip not olarak bildirilmelidir. Dergide yayınlanan yazılar için herhangi bir ücret ya da karşılık ödenmez.

Yayın kurulu yazar(lar)ın dergiye gönderdikleri yazıları değerlendirme süreci tamamlanmadan başka bir dergiye göndermeyeceklerini taahhüt ettiklerini kabul eder. İnsanlar ve hayvanlar üzerinde yapılan deneysel araştırmaların bildirildiği yazıların gereç ve yöntem bölümünde, bu araştırmanın yapıldığı gönüllü ya da hastalara uygulanan işlemler anlatıldıktan sonra kendilerinin onaylarının alındığını (informed consent) gösterir bir cümle bulunmalıdır. Yazar(lar), bu tür araştırmalarda, uluslararası alanda kabul edilen kılavuzlara (2002 yılında revize edilen 1975 Helsinki Deklarasyonu- http://www.wma.net/e/policy/b3.htm, Guide for the care and use of laboratory animals - www.nap.edu/catalog/5140.html), T.C. Sağlık Bakanlığı tarafından getirilen, 29 Ocak 1993 tarih ve 21480 sayılı Resmi gazetede yayınlanan "İlaç Araştırmaları Hakkında Yönetmelik" ve daha sonra yayınlanan diğer yönetmeliklerde belirtilen hükümlere uyulduğunu belirtmeli ve kurumdan aldıkları Etik Kurul Onayı'nın bir kopyasını göndermelidir. Metin içinde standart kısaltmalar kullanılır, bunlar ilk geçtikleri yerde açık olarak yazılır. İlaç adları kullanımında ilaçların jenerik adları Türkçe okunuşlarıyla yazılır. Ölçüm birimleri metrik sisteme uygun olarak verilir; örneğin, "mg" olarak yazılır, nokta kullanılmaz; ek alırsa („) ile ayrılır. Laboratuar ölçümleri Uluslararası Sistem (US; Systéme International: SI) birimleri ile bildirilir.

Bilimsel sorumluluk

Makalelerin tüm bilimsel sorumluluğu yazarlara aittir. Gönderilen makalede belirtilen yazarların çalışmaya belirli bir oranda katkısının olması gereklidir. Yazarların isim sıralaması ortak verilen bir karar olmalıdır. Sorumlu yazar, yazar sıralamasını "Yazar Sorumluluk ve Yayım Hakkı Devir Formu'nu" doldurarak tüm yazarlar adına kabul etmiş sayılır. Yazarların tümünün ismi makale başlığının altındaki bölümde yer almalıdır.

Yayın Ücretleri

Bu dergide yayın tamamen ücretsizdir. Yayın ücreti, başvuru ücreti, makale işleme ücreti ve bir figürün, rakamın veya tamamlayıcı verinin uzunluğuna göre ek ücret ödenmesi gerekmez. İçerik öğeleri (Editörler, Düzeltmeler, İlaveler, Geri Çekmeler, Mektuplar, Yorumlar vb.) tamamen ücretsizdir.

Etik sorumluluk

Makalelerin etik kurallara uygunluğu yazarların sorumluluğundadır. Hayvanlar üzerinde yapılan deneysel çalışmalarda, çalışma protokolünün çalışmanın yapıldığı kurumdaki hayvan deneyleri etik kurulu tarafından onaylandığı belirtilmelidir. Yazarlar etik kurul onayını makale ile birlikte göndermelidir. Eğer makalede daha önce yayımlanmış alıntı yazı, tablo, resim vs. var ise yazarlar; yayım hakkı sahibi ve yazarlarından yazılı izin alarak bu durumu makalede belirtmek zorundadır. Makalenin değerlendirilmesi aşamasında yayın kurulunun gerek görmesi halinde, makale ile ilgili araştırma verilerinin ve/veya etik kurul onayı belgesinin sunulması yazarlardan talep edilebilir.

İntihal politikası

Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi'ne (MAKÜ Sag. Bil. Enst. Derg.) Gönderilen yazılar intihal açısından değerlendirilir. Her gönderilen makale, iThenticate ve Turnitin yazılımı ile intihal için kontrol edilir. Makalenin benzerlik oranı %20'nin üzerinde ise, revize edilmesi için ilgili yazara geri gönderilir. Eğer makalenin yayınlanmasından sonra intihal kanıtlanırsa, bu makale derhal web sitesinden kaldırılır ve ilgili yazarlara makalelerinin MAKÜ Sag. Bil. Enst. Derg.'de yayınlanmasının uygun olmadığı bildirilecektir.

II- Dergiye Gönderilecek Yazı Türleri ve Özellikleri

a) Araştırma Makaleleri: Bu yazılar daha önce yayınlanmamış özgün araştırma verilerinin değerlendirildiği net anlam taşıyan bilimsel çalışmaları kapsar. Araştırma makaleleri "Öz, Giriş, Gereç ve Yöntem, Bulgular, Tartışma ve Kaynaklar" bölümlerinden oluşmalıdır. Dergide yayınlanmak üzere gönderilen araştırma makaleleri kapak sayfası hariç en fazla 20 sayfa olmalıdır. Araştırma makalelerinde kullanılacak tablo, çizim ve resim sayısı toplam 10'u geçmemelidir. Yazarlar gerek duydukları takdirde "Tartışma" bölümünden sonra "Teşekkür" bölümü açarak gerekli açıklamaları yapabilirler.

b) Derleme Makaleleri: Derleme makaleleri dergi editör/yayın kurulu tarafından "çağrılı derlemeler" başlığı altında oluşturulan alnında katkı sağlama potansiyeli olan yazıları içerir. Kaynakça bölümü en fazla 30 kaynakçadan oluşturulmalıdır. Derlemelerde kullanılacak tablo, çizim ve resim sayısı toplam 10'u geçmemelidir. Kapak sayfası hariç en fazla 20 sayfa olarak hazırlanmalıdır. Derlemelerde mutlaka "Öz, Giriş, Sonuç ve Kaynaklar" bölümleri bulunmalıdır.

c) Olgu Sunumları: Yazarların, herhangi planlanmış bir araştırmaya dayanmayan ancak karşılaştıkları yeni veya ender gözlemlenen olguların ele alındığı, bilimsel değere sahip bilgileri içeren eserlerdir. Bu eserlerde gereksiz uzatmaları önlemek amacıyla en fazla 15 kaynak kullanılmalı ve bu kaynakların güncel olmasına özen gösterilmelidir. Kapak sayfası hariç en fazla 5 sayfa olmalı; "Öz, Giriş, Olgu, Tartışma ve Kaynaklar" bölümlerinden oluşmalıdır.

d) Kısa Araştırma Raporu: Dar kapsamlı ele alınmış (sınırlı sayıda örneğin analiz edildiği çalışmalar vb.) ancak önemli ve yeni bilgiler sunan bilimsel araştırmaya dayalı makalelerdir. Kısa bildiriler araştırma makalesi formatında hazırlanmalı ve kapak sayfası hariç en fazla 10 sayfa olmalıdır. Bu eserlerde kullanılacak tablo ve şekil sayısı beşi geçmemelidir.

e) Özel Bölümler:

1. Editöre mektuplar: Dergide yayınlanan yazılara ilişkin değerlendirme ve eleştirileri içeren yazılardır. Mümkün olduğunca eleştirilen yazının yazar(lar)ınca verilen yanıtlar ile birlikte yayınlanır. Editöre mektuplar 3 sayfayı geçemez.

2. Toplantı haberleri/izlenimleri: Derginin yayın alanıyla ilgili konularda yapılmış ya da yapılacak olan bilimsel toplantıları tanıtıcı yazılardır. 1 sayfayı geçemez.

3. Dergi haberleri: Derginin yayın alanıyla ilgili konularda yayınlanmakta olan bilimsel dergileri tanıtıcı yazılardır; 1 sayfayı geçemez.

4. Web siteleri tanıtımı: Derginin yayın alanıyla ilgili konulardaki web sitelerini tanıtıcı yazılardır; 1 sayfayı geçemez.

5. Kitap/tez tanıtımı: Derginin yayın alanıyla ilgili konularda yayınlanmış bulunan kitapları/tezleri tanıtan yazılardır; 3 sayfayı geçemez.

III- Makalelerin Düzenlenmesi

Dergiye gönderilecek yazılar türlerine göre, başlık sayfası, İngilizce ve Türkçe özetler, ana metin, kaynaklar, tablo/şekil/resim bölümlerini içerir. Dergiye yayınlanması için gönderilen makalelerde aşağıdaki biçimsel esaslara uyulmalıdır: Yazı Microsoft Word programında Times New Roman yazı stilinde 12 punto büyüklüğünde, siyah renkte, 1,5 satır aralığında hazırlanmalıdır. Kenarlardan 2,5 cm boşluk bırakılmalıdır. Her sayfaya satır numarası eklenmelidir.

Anatomik terimler Latincede yazıldığı gibi kullanılmalıdır. Günlük tıp diline yerleşmiş terimler ise okundukları gibi Türkçe yazım kurallarına uygun olarak yazılmalıdır. İngilizce veya başka bir yabancı dildeki şekli ile yazılan terimler tırnak içinde belirtilmelidir. Yazının başlık sayfasında, yazının Türkçe ve İngilizce başlığı ve sayfa üstünde kullanılmak üzere boşluklar da dahil 40 karakteri aşmayacak şekilde Türkçe ve İngilizce kısa başlık önerisi bulunmalı. Çalışmaların yapıldığı klinik, anabilim dalı/bilim dalı, enstitü ve kuruluşun adı belirtilmelidir.

a) Başlık Sayfası: Gönderilen makalenin kategorisini, başlığını (Türkçe-İngilizce ve sadece ilk sözcüğün baş harfi büyük), yazarların adlarını (sadece baş harfleri büyük yazılır), çalıştıkları kurumları (rakamla dipnot olarak belirtilmeli), yazışmaların yapılacağı sorumlu yazarın adı, açık adresi, telefon ve faks numaraları ile e-posta adresini içermelidir. Sorumlu yazar yıldız (*) ile belirtilir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantının adı, tarihi ve yeri belirtilerek yazılmalıdır.

b) Ana Metin Bölümü: Yazının ana metni Öz ve Anahtar Kelimeler, Giriş, Gereç ve Yöntem, Bulgular ve Tartışma başlıkları içinde düzenlenir. Özler ve anahtar sözcükler: Türkçe ve İngilizce olmak üzere iki dilde yazılır ve yazının başlığını da içerir.

Öz 200 kelimeyi geçmemeli, çalışmanın ana noktaları olan amacını, hayvan ve örnek popülasyonunu, metodunu ve önemli sonuçlarını, çalışmadan elde edilen çıkarımı klinik olarak uygulanabilirliğini içermelidir. Yayını okumadan okuyucular için anlaşılır olmalıdır ve özet içinde kaynaklara atıf yapılmamalıdır. Türkçe ve İngilizce özetler ayrı sayfalarda yazılmalı ve özetlerin sonunda her iki dilden en az 3, en çok 5 anahtar sözcük yer almalıdır. Anahtar kelimeler Index Medicus Medical Subject Headings (MeSH)'e uygun olmalıdır. Anahtar kelimeler için www.nlm.nih.gov/mesh/MBrowser.html adresine başvurulmalıdır.

Giriş bölümünde yazının dayandığı temel bilgilere ve gerekçelere kısaca değinildikten sonra, son paragrafında amaç açık bir anlatımla yer alır. Gereç ve yöntem bölümü gerekirse araştırma/hasta/denek grubu, araçlar, uygulama ve istatistik değerlendirme gibi alt başlıklara göre düzenlenebilir. Bu bölüm çalışmaya katılmayan birisinin de rahatlıkla anlayabileceği açıklıkta yazılmalıdır. Bulgular bölümü çalışmanın sonuçlarını özetler ve temel bulgular gerekirse tablo ve şekillerle desteklenir. Tartışma bölümünde çalışmanın bulguları ilgili yurt içi ve yurt dışı çalışmaların sonuçları bağlamında tartışılır; genel bir gözden geçirmeyi değil, özgün bulguların tartışılmasını içerir. Yayın sisteme yüklenirken ana metin bölümü ana dosya olarak yüklenmelidir.

c) Teşekkür: Yazarlar çalışmalarında vermek istedikleri ek bilgiler ile katkı sağlayan destekçi kurumlara ve/veya şahıslara teşekkür yazılarını bu bölümde belirtebilirler.

d) Kaynaklar: Kaynaklar listesi alfabetik sıraya göre yazılmalıdır. Sadece yayınlanmış veya yayına kabul edilmiş kaynaklar yer almalıdır. Kabul edilmiş ancak henüz yayınlanmamış kaynaklar için "baskıda" ifadesi kullanılmalıdır. Yazarlar kaynaklar listesinde bulunan bütün kaynakların metin içinde kullanılmış olduğunu kontrol etmelidirler.

Yayındaki bütün kaynaklar kullanılmalıdır. Makale içinde referans kullanma şekline örnekler.

Metin içinde doğrudan atıf yapılırken yazar veya yazarların soyadından sonra parantez içinde kaynağın yayın yılı belirtilmelidir.

Örnekler: Bell (2005) tarafından; Nielsen ve Engberg (2006) tarafından; Doyle ve ark. (2007) tarafından

Cümlenin sonunda atıf yapıldığında ise yazar ismi ve yayın yılı parantez içinde belirtilmelidir.

Örnekler: ...bildirilmiştir (Bell, 2005); ....bildirilmiştir (Nielsen ve Engberg, 2006); .....bildirilmiştir (Doyle ve ark., 2007).

Birden çok kaynağa atıf yapılması durumunda kronolojik sıralama yapılmalıdır.

Örnekler: ….bildirilmiştir (Bell, 2005; Nielsen ve Engberg, 2006; Doyle ve ark., 2007).

Aynı yazarın aynı yıl yayınları söz konusu ise her biri "a" harfinden başlayarak küçük harflerle işaretlenmelidir.

Örnek: …. (Bell, 2005a; Bell, 2005b; Bell, 2005c …). Atıf yapılırken aşırı kaynak kullanımından kaçınılmalıdır.

Kaynaklar listesinin düzenlenmesi:

Mendeley programı kullanan yazarlar aşağıda linki verilen dergi format stilini kullanarak çalışmalarını düzenleyebilir:
https://csl.mendeley.com/styles/529990351/makusagbilensderg

Kaynaklar listesinde yazar isimleri ve yayın yılı koyu harflerle yazılmalıdır. Kaynak listesi şu şekilde hazırlanmalıdır:

i) Kaynak makale ise

Yazarların soyadları ve adlarının ilk harfi yazılmalıdır. Devamında sırasıyla makalenin yayın yılı, makalenin adı, yayınlandığı derginin açık adı, cilt, sayı ve sayfa numaraları belirtilmelidir.

Örnekler:

Cohen, N.D., Vontur, C.A., Rakestraw, P.C., 2000. Risk factors for enterolithiasis among horses in Texas. Journal of the American Veterinary Medical Association 216, 1787-1794.

Rajmohan, S., Dodd, C.E., Waites, W.M., 2002. Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. Journal of Applied Microbiology 93, 205-213.

Ono, K., Yamamoto, K., 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. International Journal of Food Microbiology 47, 211-219.

Yayınlanmak üzere kabul edilen ve DOI numarası bulunan, ancak henüz basılmamış makaleler için; makale künyesinin sonunda DOI numarası belirtilmelidir.

McGregor, B.A., Butler, K.L., 2014. The value of visual fleece assessment in addition to objective measurements in identifying Angora goats of greater clean mohair production. Small Ruminant Research, in press (DOI: 10.1016/j.smallrumres.2014.04.001).

ii) Kaynak kitap ise

Yazarların (veya editörün) soyadları ve adlarının ilk harfi yazılmalıdır. Devamında sırasıyla kitabın yayın yılı, adı, yayınevi veya yayınlayan kuruluş ve yayınlandığı yer belirtilmelidir. Kaynak, kitaptan bir bölüm ise bölüm yazarlarının isminden sonra sırasıyla kitabın yayın yılı, bölümün adı, editörün soy ismi ve adının ilk harfi, bölümün alındığı kitabın adı, yayınevi veya kuruluş, yayınlandığı yer, bölümün sayfa numaraları yazılmalıdır.

Örnekler:

Combs, G.F., 1992. The Vitamins: Fundamental Aspects in Nutrition and Health. Academic Press, San Diego.

Concannon, P.W., 1986. Physiology and Endocrinology of Cannine Pregnancy. In: Marrow, D.A. (Ed.), Current Therapy in Theriogenology. Philadelphia, W.B. Saunders Company, pp. 491-497.

Perkins, J.B., Pero, J., 2002. Vitamin biosynthesis. In: Sonenshein, A., Hoch, J., Losick, R. (Eds.), Bacillus subtilis and Its Closest Relatives: from Genes to Cells. ASM Press, Washington D.C., pp. 271-286.

Kramer, J.M., Gilbert, R.J., 1989. Bacillus cereus. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York, pp. 22-70.

iii) Kaynak bir tez ise

Tezi yazan kişinin soyadı ve adının ilk harfi koyu olarak yazılmalı, kabul edildiği yıl, tezin başlığı, tezin cinsi (yüksek lisans veya doktora), üniversitesi ve enstitüsü belirtilmelidir.

Örnek:

Bacınoğlu, S., 2002. Boğa spermasında farklı eritme süreleri ve eritme sonrasında oluşturulan soğuk şoklarının spermatolojik özelliklere etkisi. Doktora Tezi, İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, İstanbul.

iv) Kaynak internette bulunan bir web sitesi ise

Yazarların soyadları ve adının ilk harfi (Yazar adı yoksa web sitesinin veya kaynağın adı) yazılır. Daha sonra sırasıyla yılı, makalenin adı, varsa yayıncı, internet adresi ve erişim tarihi belirtilir.

Örnekler:

FDA, 2001. Effect of the use of antimicrobials in food-producing animals on pathogen load. Systematic review of the published literature. http://www.fda.gov/cvm/antimicrobial/PathRpt.pdf (Erişim 14.12.2001)

Cleveland, C.W., Peterson, D.S., Latimer, K.S., 2005. An Overview of Canine Babesiosis. Clinical Pathology. College of Veterinary Medicine, The University of Georgia: http://www.vet.uga.edu/vpp/clerk/Cleveland (Erişim 17.12.2005).

Thierry, F., 2006. Contagious equine metritis: a review. Equine Reproductive Infections: http://www.equinereproinfections.com (Erişim 07.07.2006].

FSAI, 2008. Report of the Implementation Group on Folic Acid Food Fortification to the Department of Health and Children. Food Safety Authority of Ireland: http://www.fsai.ie/assets/0/86/204/cc3c2261-7dc8-4225-bf79-9a47fbc2287b.pdf (Erişim 20.06.2008)

v) Kaynak bilimsel toplantıda sunulmuş bir bildiri ise

Yazarların soyadı ve adının baş harfinden sonra sırasıyla toplantının yılı, bildirinin başlığı, toplantının adı, toplantı yeri, bildiri kitabındaki sayfa no yazılmalıdır.

Örnekler:

Cardinali, R., Rebollar, P.G., Mugnai, C., Dal Bosco, A., Cuadrado, M., Castellini, C., 2008. Pasture availability and genotype effects in rabbits: 2. development of gastro-intestinal tract and immune function of the vermiphorm appendix. In: Proc. 9th World Rabbit Congress, Verona, Italy, 1159-1164.

Mauget, R., Legendre, X., Comizzoli, P., 1998. Assisted reproductive technology in sika deer: a program to preserve endangered deer subspecies. In: Proc. 4th Int. Deer Biology Congress, Kaspovar, 185-186.

e) Tablolar: Kullanım sırasına göre numaralandırılmalı, kısa başlıklarla ifade edilmeli ve metin içinde tablo numarası verilerek (örneğin Tablo 1) atıfta bulunulmalıdır. Tablo başlıkları tablonun üst bölümüne yazılmalıdır. Tabloda kullanılan kısaltmalar ve gerekli açıklamalar tablo altında verilmelidir.

f) Şekil ve Resimler: Metinde kullanılan fotoğraflar, grafikler ve çizimler metin içinde şekil adı ile kullanılmalıdır. Şekiller kullanım sırasına göre numaralandırmalı ve kısa başlıklarla ifade edilmeli, metin içinde şekil numarası verilerek (örneğin Şekil 1) atıfta bulunulmalıdır. Şekil başlıkları şekillerin altında yer almalıdır. Şekillerde istenilen noktaya dikkat çekmek amacıyla; üzerlerine işaret konulmalı ve başlıklardan sonra yer alacak olan şekil altı notta kullanılan işaretler belirtilerek gerekli açıklamalar yapılmalıdır.

IV- Makale Süreci (Kör hakemlik)

Makale başvurusu yalnızca online olarak http://dergipark.gov.tr/maeusabed adresi üzerinden kabul edilmektedir. Sorumlu yazar, makale ile birlikte göndereceği tüm dosyaları yukarıdaki internet adresinde bulunan yeni makale gönder ikonunu tıklayarak sisteme ekleyebilir. Yazarlar dergiye gönderi yapmadan önce kayıt olmalıdır. Kaydolduktan sonra, ana sayfadaki Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi ikonuna tıklayarak; yazım kurallarına göre düzenlenmiş bilimsel çalışmayı dergi panelindeki Makale Gönder kısmından 4 basamaklı (başlarken, yükleme, kaynaklar, önizleme&gönder) gönderi işlemini yapabilir. Gönderilen makalede ön değerlendirme aşaması sırasında yazar künyeleri, çalışmanın yapıldığı kurum, etik kurul ya da özel izin adres bilgileri gibi tanıtıcı bilgiler içermemelidir. Ön değerlendirmeden (bilimsel nitelik, dil, yazım kuralları kontrolü, İntihal kontrolü iThenticate ve Turnitin programı,) geçen bilimsel çalışmaların hakem ataması yapılır. Sorumlu yazar makalenin hangi aşamada olduğunu sistem panelindeki Süreçteki Makaleler kısmından takip edebilir. Atanan hakemlere, kör hakemlik kuralları çerçevesinde çalışmanın tam metni, şekil, tablo, grafik ve resimleri sistem üzerinden yüklenerek e-posta aracılığıyla makale değerlendirme talebi gönderilir. Hakemler e-posta aracılığıyla gönderilen linke tıklayarak talebi kabul ya da reddederler. Kabul eden hakemler, kararlarını sistem üzerinden en fazla 1 ay içinde sebeplerle birlikte yüklemelidirler. Hakemin önerdiği düzeltme var ise tekrar yazara gönderilir. İstenilen düzeltmeler 1 ay içinde tamamlanıp gönderilmediği takdirde makale otomatik olarak iptal edilecektir. Editör, makalelerin yayın değerliliği ve hakemlerin görüşlerine dayanarak yayına kabul veya red kararını verir. İstenilen düzeltmeler yapıldıktan sonra makale yazar tarafından sisteme tekrar yüklenir. Derginin gizlilik bildiriminde belirtildiği gibi, yazarların kimlik bilgileri ve e-posta adresleri hiçbir şekilde başka amaçlar için kullanılmayacaktır.

Bu dergi; bilimsel araştırmaları halka ücretsiz sunmanın bilginin küresel paylaşımını artıracağı ilkesini benimseyerek, içeriğine anında açık erişim sağlamaktadır.

Mehmet Akif Ersoy University Journal of Health Sciences Institute
INSTRUCTIONS TO AUTHORS

I- Mehmet Akif Ersoy University Journal of Health Sciences Institute General Information

Mehmet Akif Ersoy University Journal of Health Sciences Intitute (MAKU J. Health Sci. Inst.) is the publication of Mehmet Akif Ersoy University Health Sciences Institute. It is published two times annualy. The journal is a peer-reviewed scientific journal in which basic and clinical scientific articles in the field of medical sciences (veterinary, medicine, dentistry, nursing and sports sciences) are published. The language of the journal is English. Papers submitted to the journal should not have been previously published, accepted for publication or be in the process of evaluation for publication in any other journal. This rule does not apply to articles presented as bulletins in scientific meetings and whose summaries are published. In such cases, however, the name, date and place of the meeting in which the paper was presented should be notified. The format of the article should be in accordance with the rules of "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (http:/www.icmje.org/)".

On receipt of the paper by the Editorial Board, the paper is evaluated for compliance with the format rules and the authors are informed about the result in four weeks. In the event that the paper is not found to comply with the general publication principles of the journal from the standpoint of either technical characteristics or general scope, the paper is rejected. Alternatively, the author(s) may be asked to re-submit the paper in accordance with the writing requirements. Papers resubmitted are passed through a similar technical examination and, if found to comply with the rules, are passed on for peer review. The paper is sent, without the title, to two reviewers selected by the board, who then assess the paper for scientific content and format compliance. When necessary the Editorial Advisory Board can send the paper to third reviewers. The selection of reviewers is ultimately at the discretion of the editor, associate Editors and/or the editorial board. The appropriate reviewers can be selected from journal's international database of reviewers listing or, if needed; independent reviewers can be determined from inland or abroad. Thereafter the Editorial Advisory Board carries out the final editing, taking the reports of the reviewers into consideration, and, when necessary, communicating with the author(s).

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form. Measurement units are given according to the metric system; e.g. written as “mg”, no punctuation is used, in the case of extensions (,,) is used as a separator. Laboratory measurements are reported in International System Units (US; Systeme Internationale; SI).

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II- Types and Characteristics of Papers to be Submitted to the Journal

a) Research Articles: These articles are prepared in full accordance with the writing style definitions given below, in which previously unpublished original research data are evaluated. The main text section of the research articles should include (Title, Introduction Materials and Methods, Results, Discussion and Conclusion) sections and (excluding title page, bibliography, tables/figures/pictures) should not exceed 20 pages. If some parts of the research data given in these articles have previously been discussed in another paper, this must be notified without fail when sending the paper and, in addition, reference should be made to the relevant paper within the bibliography.

b) Review Articles: Review Articles should cover subjects falling within the scope of the journal which are of active current interest. They may be submitted or invited. Invited reviews will normally be solicited by the Review's Editor, but suggestions for appropriate review topics may be sent to editor.

c) Case Reports: These are articles which present and discuss the characteristics of one or more cases which have special features and scientific importance from the clinical evaluation, observation or other standpoint. Case presentations include the title page, summary, main text (includes introduction, case and discussion), bibliography, table/figure/picture sections; subtitles in the main text are organised according to the text content. Abstracts of the

case presentations should have 150 words. The main text (excluding title page, bibliography, table/figure/picture) should not exceed 10 pages.

d) Brief Reports: These are articles in which original ideas dealing with important theoretical or practical problems related to a specific subject are presented and discussed. Original articles include a title page, summary,

main text, bibliography, table/figure/picture sections; subtitles in the main text are organised according to the text content. The main text of original articles (excluding title page, bibliography, table/figure/picture) should not exceed 10 pages.

e) Special Sections:

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2. Meeting news/notes: These articles introduce scientific meetings held or to be held on subjects within the scope of the journal. The paper may not exceed 1 page.

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4. Introduction of websites: These articles introduce websites relevant to the scope of the journal. These articles may not exceed 1 page.

5. Book/Thesis Section: These articles introduce books/theses published on subjects related to the scope of the journal and may not exceed 3 pages.

III- Preparation of Manuscripts

Papers to be submitted to the journal include the sections of title page, abstract, main text, references and tables/figures/pictures. Articles submitted for publication in the journal should follow the following formal principles: The text should be prepared in Microsoft Word program in Times New Roman font style with a font size of 12 font, black and 1.5 line. All side of the paper, page margins should be as 2.5 cm. Line numbers should be added to the beginning of the page.

Anatomical terms should be used as written in Latin. Running title (not exceed 40 characters) of the manuscript should add to title page. The name of the clinic, department / science, institute and institution should be stated.

a) Title Page: should contain the category, the title (only first letter capital), the names of the authors (only the first letters capital), the institution (s) where they work (indicated with numbered footnotes), corresponding author (address, phone, fax numbers and e-mail address). Corresponding author is indicated by an asterisk (*). If the article was previously presented at a scientific meeting, the name, date and place of the meeting must be stated.

b) Main Text: The main text of the paper is organised under the subtitles of Abstract and Keywords, Introduction, Materials and Methods, Results and Discussion.

Abstract and Keywords: This is written in two languages, Turkish and English, and also includes the title of the paper. The abstract is consists of 200 words. The abstract should bring out the main points of the manuscript and should include the following information: objective, the animals or sample population involved, design, the materials and methods used, the main results, a brief conclusion and clinical relevance, where applicable. They should be comprehensible to readers before they have read the paper, and abbreviations and reference citations should be avoided. At the end of the abstract, at least 3, at most 5 keywords in both languages are included.

In the introduction, following a brief statement of basic information and justifications which constitute the basis of the paper, the objective is clearly given in the last paragraph. If necessary, the “method” section may

be organised according to sub-titles such as research/patient/ test group, instruments, application and statistical analysis. This section should be written with clarity so that a person not involved in the study may easily understand. Results summarize the findings of the study and, when necessary, basic findings are supported with tables and figures. In the discussion section, the findings of the study are discussed in the light of relevant national and international studies; this section includes discussion of original findings, not a general review.

c) Acknowledgements: When considered necessary, author(s) may add brief acknowledgements in a few sentences to those whose contributions to the paper are not at author level but deserve to be mentioned. Here, the contributions of those acknowledged (e.g. financial or equipment aid, technical support etc) are clearly stated (e.g. "scientific counseling", "editing of the draft", "data collection", "participation in clinical research" etc).

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All citations in the text should refer to: the year of publication of the reference should be indicated in parentheses after the surname of the author or authors.

Examples: Bell (2005), Nielsen and Engberg (2006), Doyle et al. (2007) were indicated that.....

The name of the author and the year of publication should be stated in parentheses at the end of the sentence.

Examples: ...were detected as 23% of the samples (Bell, 2005); ....were detected as 23% of the samples (Nielsen and Engberg, 2006); ...were detected as 23% of the samples (Doyle et al., 2007).

In case of more than one reference, references should be arranged chronologically.

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i) Examples of journal articles:

Cohen, N.D., Vontur, C.A., Rakestraw, P.C., 2000. Risk factors for enterolithiasis among horses in Texas. Journal of the American Veterinary Medical Association 216, 1787-1794.

Rajmohan, S., Dodd, C.E., Waites, W.M., 2002. Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. Journal of Applied Microbiology 93, 205-213.

Ono, K., Yamamoto, K., 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. International Journal of Food Microbiology 47, 211-219.

For articles that are accepted for publication and have a DOI number but not yet published; DOI number must be specified at the end of the article.

McGregor, B.A., Butler, K.L., 2014. The value of visual fleece assessment in addition to objective measurements in identifying Angora goats of greater clean mohair production. Small Ruminant Research, in press (DOI: 10.1016/j.smallrumres.2014.04.001).

ii) Books:

Combs, G.F., 1992. The Vitamins: Fundamental Aspects in Nutrition and Health. Academic Press, San Diego.

Concannon, P.W., 1986. Physiology and Endocrinology of Cannine Pregnancy. In: Marrow, D.A. (Ed.), Current Therapy in Theriogenology. Philadelphia, W.B. Saunders Company, pp. 491-497.

Perkins J.B., Pero, J., 2002. Vitamin biosynthesis. In: Sonenshein, A., Hoch, J., Losick, R. (Eds.), Bacillus subtilis and Its Closest Relatives: from Genes to Cells. ASM Press, Washington D.C., pp. 271-286.

Kramer, J.M., Gilbert, R.J., 1989. Bacillus cereus. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York, pp. 22-70.

iii) Thesis:

Bacınoğlu, S., 2002. Boğa spermasında farklı eritme süreleri ve eritme sonrasında oluşturulan soğuk şoklarının spermatolojik özelliklere etkisi. Doktora Tezi, İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, İstanbul.

iv) Web site or author is an institution:

FDA, 2001. Effect of the use of antimicrobials in food-producing animals on pathogen load. Systematic review of the published literature. http://www.fda.gov/cvm/antimicrobial/PathRpt.pdf (Accessed: 14.12.2001)

Cleveland, C.W., Peterson, D.S., Latimer, K.S., 2005. An Overview of Canine Babesiosis. Clinical Pathology. College of Veterinary Medicine, The University of Georgia: http://www.vet.uga.edu/vpp/clerk/Cleveland (Accessed: 17.12.2005).

Thierry, F., 2006. Contagious equine metritis: a review. Equine Reproductive Infections: http://www.equinereproinfections.com (Accessed: 07.07.2006).

FSAI, 2008. Report of the Implementation Group on Folic Acid Food Fortification to the Department of Health and Children. Food Safety Authority of Ireland:http://www.fsai.ie/assets/0/86/204/cc3c2261-7dc8-4225-bf79-9a47fbc2287b.pdf (Accessed: 20.06.2008).

v) Paper presented at a scientific meeting

Cardinali, R., Rebollar, P.G., Mugnai, C., Dal Bosco, A., Cuadrado, M., Castellini, C., 2008. Pasture availability and genotype effects in rabbits: 2. development of gastro-intestinal tract and immune function of the vermiphorm appendix. In: Proc. 9th World Rabbit Congress, Verona, Italy, 1159-1164.

Mauget, R., Legendre, X., Comizzoli, P., 1998. Assisted reproductive technology in sika deer: a program to preserve endangered deer subspecies. In: Proc. 4th Int. Deer Biology Congress, Kaspovar, 185-186.

e) Tables: Each table is printed on a separate page and numbered according to the sequence of referral within the text (Table 1). Each table has a title and, when necessary, explanations are given under the table (e.g. abbreviations given in the table). Each table should be understandable without need for referral to the text. Each table should be referred to in the text..

f) Figures and Pictures: Figures should be numbered according to the order of use and should be expressed with short titles.Figures should be numbered in the text (Figure 1). Letters, numbers and symbols within the figure should be clear and readable when downsized for printing.Each figure should be referred to in the text..

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Dişi Sıçanlarda Resveratrol'ün Metotreksat ile İndüklenen Renal Toksisiteye Karşı Nefroprotektif Etkisi 123-133
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MAKU J. Health Sci. Inst. 2022, 10(2): 103-107.
doi: 10.24998/maeusabed.1061278

Mehmet Akif Ersoy University
Journal of Health Sciences Institute
http://dergipark.gov.tr/maeusabed

Research Article / Araştırma Makalesi

Vitamin D Levels in Cats Infected with Feline Herpesvirus Type-1

Feline Herpesvirüs Tip-1 ile Enfekte Kedilerde Vitamin D Düzeyleri

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Abstract: In this study, it was aimed to measure vitamin D levels in Feline Herpesvirus Type-1 (FeHV-1) infected cats and thus to determine its relationship with the disease activity. The animal material of this study was composed of 20 cats of different breeds, ages and genders, which showed clinical symptoms of the disease and were diagnosed with Feline Herpesvirus infection by rapid test. The control group of the study consisted of 10 cats of different breeds, ages and genders, which were healthy according to the results of routine physical examination, laboratory works, which were brought for the purpose of vaccination, and which were determined to be Feline Herpesvirus Type-1 antigen negative with the rapid test. Blood samples were taken from cats in both groups and 25-hydroxy vitamin D3 levels were determined by the fluorescent immunoassay method. The mean standard deviation values of 25 hydroxyvitamin D3 levels in FeHV-1 infected cats and healthy cats were found to be 33.30 and 64.70 ng/ml, respectively. FeHV-1 infected cats showed a significant decrease in serum vitamin D levels compared to healthy cats in the control group. As a result, vitamin D deficiency may have an effect on the formation of the disease.

Keywords: Cat, Feline Herpesvirus Typr-1, Vitamin D.

Öz: Bu çalışmada, Feline Herpesvirüs Tip-1 (FeHV-1) ile enfekte kedilerde D vitamin düzeylerinin ölçülmesi ve böylelikle hastalık aktivitesi ile olan ilişkisinin belirlenmesi amaçlandı. Bu çalışmanın hayvan materyalini hastalığa ait klinik semptom gösteren ve yapılan hızlı test ile Feline Herpes Virus enfeksiyonu tanısı konulan değişik ırk, yaş ve cinsiyette 20 adet kedi oluşturdu. Çalışmanın kontrol grubunu ise aşılama amacı ile getirilen rutin fizik muayene ile laboratuvar muayenesi sonuçlarına göre sağlıklı olan ve yapılan hızlı test ile Feline Herpesvirus Tip-1 antijen negatif olarak belirlenen değişik ırk, yaş ve cinsiyette 10 adet kedi oluşturdu. Her iki gruptaki kedilerden alınan kan örneklerinden florösan immunoassay yöntemi ile 25 hidroksivitamin D3 düzeyleri belirlendi. FeHV-1 ile enfekte kediler ve sağlıklı kedilerde 25 hidroksivitamin D3 seviyelerine ait ortalama standart sapma değerleri gruplara göre sırası ile 33,30 ve 64,70 ng/ml olarak saptandı. FeHV-1 ile enfekte kedilerde, serum vitamin D düzeylerinde kontrol grubundaki kedilere göre önemli oranda düşme şekillenmiştir. Sonuç olarak D vitamini eksikliğinin hastalığın oluşumu üzerine etkisi olabilir.

Anahtar Kelimeler: Kedi, Feline Herpesvirüs Tip-1, D vitamini.

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Geliş tarihi / Received : 03.02.2022
Kabul tarihi / Accepted: 13.06.2022

Introduction

Feline Herpesvirus Type-1 (FeHV-1) is a highly contagious infectious agent affecting the upper respiratory tract and eyes in cats (Stiles, 2003; Maggs, 2005). The virus causes intense infection in the conjunctiva and cornea, as well as upper respiratory tract problems. Animals that survive the primary infection usually become latently infected in their trigeminal ganglia. Most recovered animals carry the agent throughout their lives and continue to shed it (Gaskell et al., 2007; Townsend et al., 2013; Jubb et al., 2016; Thomasy et al., 2016). It is suggested that the cause of 50-75% of upper respiratory tract diseases in cats is FeHV-1 (Townsend et al., 2013).

To cite this article: DEMİROĞLU EDY, KARAKURUM MÇ. (2022). Vitamin D Levels in Cats Infected with Feline Herpesvirus Type-1. MAKU J. Health Sci. Inst., 10(2), 103-107.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

FeHV-1 infections have a multifactorial character. The occurrence and severity of the disease depend on many factors such as virulence, the transmission route of the infection, the susceptibility of the animal, the dose of the virus, the secondary infections that may develop, the age of the animal, and the stress and environmental conditions (Kawaguchi et al., 1995; Stiles, 2003; Maggs, 2005).

Vitamin D belongs to the group of fat-soluble vitamins. Vitamin D has 2 different functions:

1. It has paracrine or autocrine effects as a local cytokine in some tissues. Vitamin D, which protects the organism from infectious agents, is also synthesized from monocytes and macrophages.

2. Another function of Vitamin D is to exert a hormone-like effect on the circulation as a result of further development. In case of insufficient amount in the circulation, it stimulates the kidneys through PTH, and as a result, the production of 1,25(OH)2 increases and its normal levels in the circulation are achieved (Özkan et al., 2009). Vitamin D deficiency may occur due to insufficient exposure to sunlight, insufficient dietary intake, and malabsorption. In vitamin D deficiency, problems such as cardiovascular diseases, chronic musculoskeletal pain, rickets, osteoporosis, obesity, Type I and Type II Diabetes Mellitus (DM), microalbuminuria, some cancer types and autoimmune diseases occur (Autier et al., 2014). While most animals synthesize vitamin D in the skin with sunlight; This event rarely occurs in cats and dogs. Direct sunlight contact with the skin is required for synthesis (Mc Dowell, 2000).

In this study, it was aimed to investigate the vitamin D levels in naturally infected cats with FeHV-1, to compare the amounts in sick animals and healthy animals and to evaluate the possible role of deficiency of this vitamin in the formation of the disease.

Materials and Methods

This research was carried out based on the permission of Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee, dated 16.01.2019 and numbered 484.

Owned cats brought to private veterinary clinics in Antalya were included in the study. Kittens and adult cats that were not vaccinated with FeHV-1 within 21 days were used in the study. Informed consent form was obtained from the cat owners. The anamnesis, age, gender, breed, and the number of days they have been sick for the cats brought to the clinic with suspected FeHV-1 clinical findings were recorded.

FHV Ag (Feline Herpesvirus) and FCV Ag (Feline Calicivirus) rapid test kits were used in cats suspected of FeHV-1 (wheezing in the upper respiratory tract, weight loss, weakness, stagnation, fever, cough, ocular discharge, salivation) in accordance with the recommended procedures and only those positive for FHV Ag and negative for FCV Ag were included in the study (Diseased group n: 20 cats). The control group (n:10) consisted of cats that were negative in all tests. Cats that did not show any suspicious clinical symptoms of FHV and were positive for FCV Ag together with FHV Ag were not included in the study.

During the study, test kits (Savant, Bejing Savant Biotechnology Co, Ltd 25-OH-D) were used for the quantitative determination of 25(OH)D levels in serum plasma or whole blood samples. Measurements were performed with a Savant fluorescent immunoassay device using the chromatographic method.

Statistical analysis

Statistical package software (Minitab 16.1.1, 2011) was used for statistical comparison of the data. Since the data did not show normal distribution as a result of the normality test, non-parametric Mann-Whitney statistical analysis method was applied.

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Table 1. Vitamin D levels in the Diseased and Control Group.

Groups	Number of cats	Median	
Control	10	64.70	P<0.005
Diseased	20	33.30	

Results

The age of the FeHV-1 infected cats used in the study was minimum 1.5 months and maximum 3 months, and this group consisted of 11 female and 9 male cats. While the age of the cats in the control group was minimum 1.5 months and maximum 7 months, this group consisted of 3 male and 7 female cats.

Swap samples were taken from cats with classical rhinotracheitis, rhinitis, chronic sinusitis, corneal ulcer, stromal keratitis, keratoconjunctivitis sicca and applied in accordance with the high fever, stagnation, anorexia, depression, sneezing and conjunctivitis were determined in all FeHV-1 positive cats.

Vitamin D levels in the diseased and control group was given in Table 1. When compared with the control group Vitamin D levels in diseased group was significantly lower (P<0.005).

Discussion

It is suggested that the cause of 50-75% of upper respiratory tract diseases in cats is FeHV-1 (Townsend et al., 2013). Many studies conducted in the past years have shown the prevalence of FeHV-1 in domestic and wild cat populations (Di Martino et al., 2007). Although vaccination for FeHV-1 does not provide complete protection against infection, it is known that it reduces the shedding time of the virus and the amount of shed virus and positively affects the prognosis (Weigler et al., 1997; Maggs, 2005). For this reason, it is known that in areas where the cat population is high and cat births cannot be controlled, practices such as increasing the population immunity level with routine vaccination and disinfection may contribute to limiting the increase in the rate of transmission, although they do not completely eliminate the infection (Berger et al., 2015). In addition, many previous studies have reported that FeHV-1 infection causes coinfection with other viral and bacterial agents (Burns et al., 2011; Filoni et al., 2012; Berger et al., 2015; Litster et al., 2015).

According to many studies in the past years, vitamin D is a group of sterols that enable hormonal functions to take place. Apart from vitamin metabolism, vitamin D has a very important place for vital functions in the body (Dusso et al., 2005; Holick, 2008; Jussila et al., 2013).

Vitamin D is also associated with many different diseases and inflammations. The presence of VDR in inflammatory cells may also explain the importance of vitamin D's effect on the immune system. In cases of vitamin D deficiency, a decrease in T cell response occurs (Nicholson et al., 2012). Vitamin D appears to have a direct effect on the response and development of T cells (Ulitsky et al., 2011) from T cells; Th1 (T1 helper) stimulates proinflammatory cytokine production and provides a strong immune response. Th2 (T2 helper) is involved in the release of anti-inflammatory cytokines (Özkan et al., 2011; Raman et al., 2011). Vitamin D inhibits the proliferation of Th1 cells and, together with interferon gamma, suppresses the formation of proinflammatory cytokines such as interleukin-2 (Lim et al., 2005; Nerich et al., 2011). Proinflammatory cytokines play a major role in the pathogenesis of many diseases (Hassan et al., 2013; Özkan et al., 2011).

Possible causes of low vitamin D in cats include; low or decreased dietary intake and the effects of some drugs. While some drugs, such as glucocorticoids, affect vitamin D metabolism in humans, it has been shown not to alter vitamin D metabolism in dogs. However, it is unclear

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whether many commonly used drugs affect vitamin D metabolism in cats, and more research is needed on this subject (Kovalik et al., 2012a). In addition, some studies have suggested that vitamin D may be a negative acute phase reactant (Waldron et al., 2013).

Although studies in cats show low serum 25(OH)D levels in diseases such as inflammatory bowel disease, gastrointestinal lymphoma, and mycobacterial infection, little is known about the relationship between vitamin D level and disease prognosis in cats (Lalor et al., 2012, 2014). Low serum concentrations of 25(OH)D have been shown to be associated with an increased risk of death in sick cats (Titmarsh et al., 2015). In addition, low vitamin D level has been shown to be associated with a weaker response to prednisolone treatment in atopic skin disease in dogs (Kovalik et al., 2012b).

It has been reported that FIV-infected cats have significantly lower vitamin D levels than cats in the healthy control group. However, further investigation of the relationship between vitamin D levels and long-term prognosis in FIV-infected cats has been recommended (Titmarsh et al., 2015). In this study, it was determined that the vitamin D levels of cats infected with FeHV-1 were statistically significantly lower than those of healthy cats. Considering the known immunomodulatory effects of vitamin D metabolites, a relationship between Feline Herpesvirus Type-1 infection and possible low vitamin D levels is possible. However, more studies are needed on this subject and the effects of Vitamin D level on the prognosis of the disease.

FeHV-1 infected cats showed a significant decrease in serum vitamin D levels compared to healthy cats in the control group. As a result, vitamin D deficiency may have an effect on the formation of the disease. It can be said that by measuring vitamin D levels in FeHV-1 infected cats or healthy cats, adding the necessary supplements for protection and treatment in sick ones in case of deficiency will be beneficial.

More studies are needed to investigate the effects of vitamin D levels on susceptibility to diseases and treatment outcomes.

Acknowledgments

This study was supported by Burdur Mehmet Akif Ersoy Scientific Research Projects Commission (Project No: 567-YL-19).

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MAKU J. Health Sci. Inst. 2022, 10(2): 108-115.
doi: 10.24998/maeusabed.1130028

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Research Article / Araştırma Makalesi

Association Between Serum Netrin-1 Level and Obesity-related Markers in Obese Subjets

Obez Bireylerde Serum Netrin-1 Düzeyi ve Obezite ile İlgili Belirteçler Arasındaki İlişki

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Abstract: Netrins are important signaling proteins that guide both neural and vascular development. Netrin-1 regulates many physiological processes such as cell proliferation, adhesion and migration. The aim of this study was to determine the association between serum netrin-1 level and biochemical parameters in obese subjects. Serum samples were collected from obese (n=15) and control (n=30) subjects. The serum netrin-1 levels were evaluated by ELISA. Variable data were compared by Mann Whitney U test and correlation was estimated by Spearman correlation analyses. The level of circulating netrin-1 protein was determined to be significantly lower in obese group compared to controls. The median value was 844 pg/mL in the control group and 338 pg/mL in the obese group ($p<0.0001$). Serum netrin-1 level was negatively correlated with HbA1c, LDL, cholesterol and triglyceride ($P< 0.01$). In addition, ROC curve analysis indicated that netrin-1 level could define the presence of obesity with AUC value of 0.8667 (95% Cl=0.7249-1.000; $P<0.0001$). Our study suggests that netrin-1 secretions are significantly reduced in obese subjects and negatively correlated with obesity markers. Therefore, netrin-1 can be evaluated as a possible potential biomarker for obesity.

Keywords: Obesity, Netrin-1, ELİSA, Biomarker.

Öz: Netrinler, hem nöral hem de vasküler gelişime rehberlik eden önemli sinyal proteinleridir. Netrin-1 hücre çoğalması, yapışması ve göçü gibi birçok fizyolojik süreci düzenler. Bu çalışmanın amacı obez bireylerde serum netrin-1 düzeyi ile biyokimyasal parametreler arasındaki ilişkiyi belirlemektir. Obez (n=15) ve kontrol (n=30) bireylerden serum örnekleri alındı. Serum netrin-1 seviyeleri ELISA ile değerlendirildi. Değişken veriler Mann Whitney U testi ile karşılaştırıldı ve korelasyon Spearman korelasyon analizleri ile belirlendi. Obez grupta serumda netrin-1 protein seviyesinin kontrollere göre anlamlı derecede düşük olduğu belirlendi. Ortanca değer kontrol grubunda 844 pg/mL ve obez grupta 338 pg/mL idi ($p<0,0001$). Serum netrin-1 düzeyi HbA1c, LDL, kolesterol ve trigliserit ile negatif korelasyon gösterdi ($P< 0,01$). Ayrıca, ROC eğrisi analizi, netrin-1 seviyesinin 0,8667 AUC değeri ile obezite varlığını tanımlayabildiğini gösterdi (%95 Cl=0,7249-1,000; $P<0,0001$). Çalışmamız, netrin-1 salınımının obez bireylerde önemli ölçüde azaldığını ve obezite belirteçleri ile negatif korele olduğunu göstermektedir. Bu nedenle netrin-1, obezite için olası bir potansiyel biyobelirteç olarak değerlendirilebilir.

Anahtar Kelimeler: Obezite, Netrin-1, ELİSA, Biyobelirteç.

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Geliş tarihi / Received : 13.06.2022 Kabul tarihi / Accepted: 18.07.2022

Introduction

Nutrition; it is the adequate intake of the nutrients required by our body in order to protect human health, improve the quality development. Today, modern life conditions cause excessive energy intake due to overnutrition, while finding solutions to health problems related to malnutrition (Cayir et al., 2011). The World Health Organization defines obesity as "the increase of fat cells to levels that adversely affect human health". Obesity is a public health problem that has become common

To cite this article: AYDIN ACAR Ç, AYDIN N, PEHLİVANOĞLU S, KOK M. (2022). Association Between Serum Netrin-1 Level and Obesity-related Markers in Obese Subjets. MAKU J. Health Sci. Inst., 10(2), 108-115.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

in developed societies in recent years (Bakhshi et al., 2008). The age, gender, nutritional habits, sociocultural structure, daily physical activity and genetic structure of the individual play an active role in the diagnosis of obesity. It is very important to know the factors that cause obesity, to solve the health problems caused by obesity and to take pre-disease precautions (Deepa et al., 2009).

Netrin originates from the Sankrit word 'netr' and means 'guiding, leading' (Gorur et al., 2018). Netrins are a highly conserved family of proteins that direct axons to the ventral midline of the nervous system during embryogenesis (Rajasekharan and Kennedy, 2009). The first member of this family (UNC-6) was identified in a nematode, *Caenorhabditis elegans* (Yim et al., 2018). The gene organization was the first reported netrin UNC-5 (Rajasekharan and Kennedy, 2009). The first mammalian homologue of UNC-6 was discovered in 1994, and it has been reported to be a vital guide for the commissural axon found in the rodent spinal cord (Moore and Fisher, 2012). For mammals, five netrins are defined (netrin-1, -3, -4, -G1 and -G2). While netrin-1, netrin-3, netrin-4 are secreted from the membrane; netrin-G1 and netrin-G2 are membrane bound to the plasma membrane by two glycosylphosphatidylinositol (GPI) (Rajasekharan and Kennedy, 2009). Netrin has two identified receptors, DCC (deleted receptors in colorectal cancer) and UNC5 (uncoordinated 5). Netrin-1 is expressed in many tissues such as pancreas, lung, liver, intestines, spleen, kidney and vascular endothelial cells, especially in the central nervous system (Wang et al*.,* 1999).

The fact that netrin-1 receptors also have been identified in other cells in addition to neurons has strengthened the hypothesis that this protein may also have roles outside the central nervous system. Recent studies have revealed that netrin-1 is involved in many physiological processes from angiogenesis to inflammation (Rajasekharan and Kennedy, 2009).

In addition to its axonal guidance role in the central nervous system, new studies have shown that netrin also plays a role in cancer regulation. The upregulation of netrin-1 levels in tumors has brought up the possibility of being a biomarker that can be used in the early diagnosis of cancer. Recent studies have found strikingly increased levels of netrin-1 in blood samples from patients with kidney, liver, prostate, breast, menangioma, and glioblastoma (Mehlen and Furne, 2005; Ramesh et al., 2011). It has also been shown that netrin-1 plays a role in the development and formation of tissues other than nerve cells in cardiovascular and kidney diseases (Lu et al., 2004; Park et al., 2004; Ramesh, 2012; Wang et al., 2009).

In this study, it was aimed to determine the netrin-1 level in serum samples of obese subjects and to compare them with healthy controls, and accordingly to clarify the significant relationship between obesity and altered netrin-1 concentration.

Materials and Methods

Experimental design

Serum samples were obtained from obese (n=15) and control (n=30) groups. Human netrin-1 protein level in this samples was measured using ELISA (Enzyme-Linked Immuno Sorbent Assay). The determined netrin-1 concentrations were compared with the biochemical parameters (fasting blood glucose; HgA1c, glycosylated hemoglobin; HOMA-IR, insulin resistance test; CRP, C-Reactive protein; HDL, high density lipoprotein; LDL, low density protein; TG, total triglyceride; TC, total cholesterol; TSH, thyroid stimulating hormone; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; sedimentation; creatinine) of the obese and control groups.

Subjects

Blood samples were obtained from obese subjects and healthy controls who met the research criteria between August-2019-August-2021 in the Internal Medicine Clinic of Health Sciences University Antalya Training and Research Hospital (Antalya, Turkey). The demographic informations of

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

patients were obtained by a trained clinician. Each test sample was recruited according to the inclusion and exclusion criteria. Inclusion criteria; BMI>30, absence of obesity-related chronic disease and exclusion criteria; presence of malignancy, presence of active infection, diabetes mellitus, peripheral vascular disease, atherosclerotic heart disease, hyperlipidemia, hypertension, kidney failure, smoking, BMI <30. This study was approved by the Health Sciences University Antalya Training and Research Hospital Clinical Research Ethics Committee [2019-215/04.07.2019]. All patients included in the study signed an informed consent.

Blood collection and processing

Whole blood samples were collected in serum vacuum tubes with clot activator and gel separator (BD Vacutainer). These samples were centrifuged for 10 minutes at 2500 x g to separate the serum. Finally, the obtained serum specimens were aliquoted and stored at -80 °C until experimental analysis.

Detection of serum Netrin-1 levels by ELISA

Serum netrin-1 levels were quantified in duplicate with specific Human Netrin-1 ELISA kit (#E1277Hu, Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's instructions. The minimum detectable human netrin-1 level was 10 pg/mL. After adding the stop solution in the final step, the optical density (OD) value of almost each test well was determined at 450 nm using a microplate reader (MultiscanGO, Thermo Fisher Sci.), Results were expressed as pg/mL.

Statistical analysis

Statistical analysis and graphical presentation were performed with Graph Pad Prism 9.0.2 software version. Data were expressed as median values or mean ± standart deviation. Kolmogorov-Smirnov test was used to evaluate the distribution of variables. Mann Whitney U test was used for comparison of continuous variables. Correlation analyses were performed using the Spearman correlation test. ROC curve analysis was performed to determine the optimal cut-off values of serum netrin-1 with maximum specificity and sensitivity. p values < 0.05 were considered statistically significant.

Results

Demographic and Biochemical Findings

The obese group were consisted of 11 women (73%), 4 men (27%), and the control group were consisted of 23 women (77%), 7 men (23%). The mean age was 41.7 and 33.8 years in the obese and control groups, respectively.

By comparing the biochemical parameters of the obese and control groups, it was determined that HbA1c, CRP, LDL, TC and TG values were significantly higher in the obese group compared to the control group (p<0.0001, p:0.0002, p:0.0044, p:0.0299, p:0.0212, respectively). Fasting Insulin, HOMA-IR, Sedimentation, Creatinine, ALT, AST, HDL, BUN and TSH values were also compared between the obese and group, but no statistically significant difference was recorded (p:0.1034, p:0.1147, p:0.2404, p:0.3593, respectively). Demographic and biochemical findings of the obese and control groups are given in Table 1.

Serum Netrin-1 Level in Obese and Control Groups

Circulating netrin-1 levels of the obese and control groups were calculated and analyzed according to ELISA data. The minimum value was 224 pg/mL and the maximum value was 2274 pg/mL in the obese group, nevertheless the minimum value was 611.2 pg/mL and the maximum value was 4073 pg/mL in the control group. The median value of serum netrin-1 level was 338 pg/mL in the obese group, and 844 pg/mL in the control group. According to these results, serum netrin-1 level was statistically significantly decreased in the obese group compared to the control group (p<0.0001). The graphical representation of serum netrin-1 concentrations of the obese and control groups were shown in Figure 1.

To cite this article: AYDIN ACAR Ç, AYDIN N, PEHLİVANOĞLU S, KOK M. (2022). Association Between Serum Netrin-1 Level and Obesity-related Markers in Obese Subjets. MAKU J. Health Sci. Inst., 10(2), 108-115.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Table 1. Demographic and biochemical characteristics

Parameters	*Obese Group (Mean)*	*Control Group (Mean)*	*P*
Age	41,73	33,83	**0,0376***
Fasting insulin	12,65	6,350	0,1034
HbA1c	6,050	5,204	**<0,0001****
HOMA-IR	3,565	1,557	0,1147
CRP	11,94	1,220	**0,0002*****
Sedimentation	7,000	4,889	0,2404
Creatinine	0,7350	0,8000	0,3593
ALT	23,50	22,24	0,8097
AST	19,0	19,0	0,6233
HDL	58,77	65,90	0,1592
LDL	135,5	104,5	**0,0044****
TC	222,0	189,8	**0,0299***
TG	139,1	96,10	**0,0212***
BUN	10,71	12,07	0,3472
TSH	1,947	2,192	0,5639

HgA1c, glycosylated hemoglobin; HOMA-IR, insulin resistance test; CRP, C-Reactive protein; HDL, high density lipoprotein; LDL, low density protein; TG, triglyceride; TC, cholesterol; TSH, thyroid stimulating hormone; BUN, blood urea nitrogen; ALT, alain aminotransferase; AST, aspartate aminotransferase, *P<0.1;**P<0.01; ***P<.0.001; ****P<0.0001

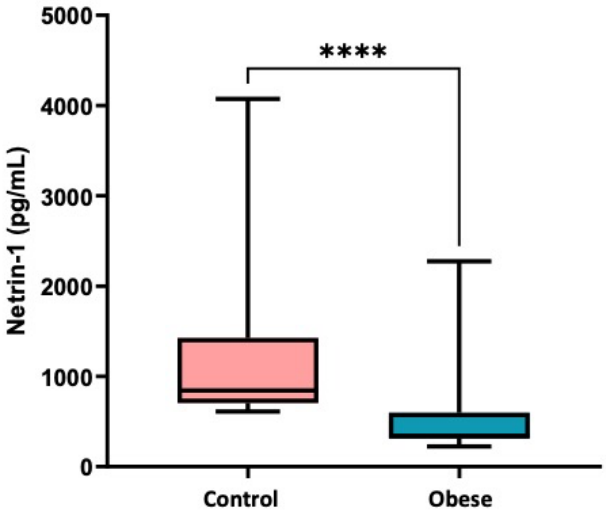

Figure 1. Distribution of serum netrin-1 level in obese and healthy control groups. Serum netrin-1 level was statistically significantly decreased in the obese group compared to the control group (p<0.0001). The center box and the middle line of each graph represent values from the bottom to the upper quartile (25th - 75th percentile) and the median, respectively. Horizontal lines represent the minimum and maximum values.

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst

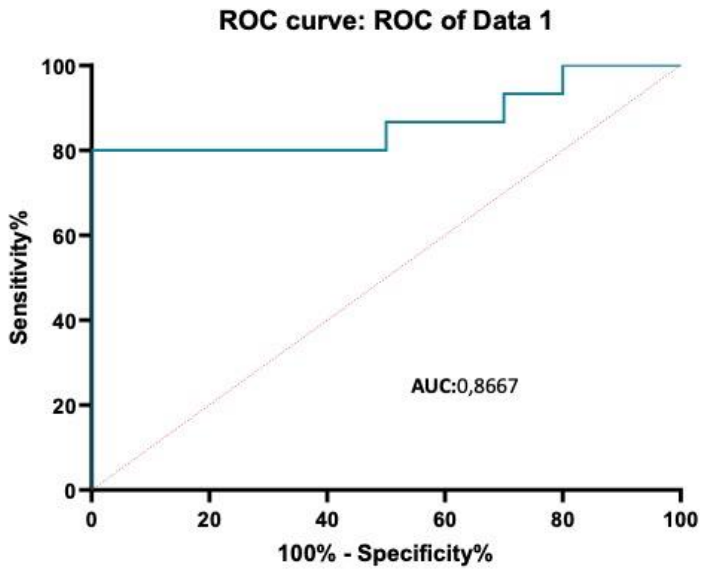

Figure 2. The Receiver Operating Characteristic (ROC) curve of serum levels of Netrin-1 to differentiate of obese from control. AUC: 0,8667 (95% Cl=0.7249-1.000; P<0.0001).

ROC curve analysis was used to measure of the usefulness and analyze diagnostic accuracy of the serum netrin-1 ELISA test that discriminate different two groups (Figure 2). The AUC (area under the ROC curve) was 0.8667 (95% Cl=0.7249-1,000; P<0.0001). The threshold value for serum netrin-1 was <624.2 pg/mL, with 80% sensitivity and 93.3% specificity in distinctive obesity.

Serum Netrin-1 Level and Biochemical Parameters

Spearman correlation was used to determine the association between serum netrin-1 level and biochemical parameters (Table 2). A negative correlation was found between serum netrin-1 level and HbA1c, LDL, TG (r= -5024; p: 0.0025, r=-0.3696; p: 0.0343, r=-0.3886; p: 0.0231). No significant correlation was determined between serum netrin-1 level and age, fasting insulin level, HOMA-IR, CRP, sedimentation, HDL, TC, TSH, BUN, ALT, AST and creatinine values.

Discussion

Obesity is an important worldwide public health problem that causes morbidity and needs novel therapeutic approaches and accepted international consensus for its treatment. It becomes very complicated with type 2 diabetes, liver diseases, cardiovascular diseases, hypertension, respiratory problems and some types of cancer (Mayoral et al., 2020).

Netrin is a family of highly conserved extracellular proteins with important roles in the central nervous system (Rajasekharan and Kennedy, 2009). Since the angiogenic, regenerative and anti-inflammatory properties of netrin-1 have been reported before, it was estimated that netrin-1 may be have important roles in various biological processes besides the central nervous system. In this context, the aim of this study is to reveal the potential association between obesity and netrin-1 concentrations.

A total of 45 volunteers, 15 of whom were obese and 30 were controls, were included in our study. Serum netrin-1 level was evaluated by ELISA method. In our study, it was clarified that serum netrin-1 level was statistically significantly lower in the obese group compared to the control group. In addition to these findings, a negative correlation was found between serum netrin-1 level and HgA1c, LDL, TG.

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Table 2. The correlation of serum netrin-1 and biochemical parameters of the obese and control groups.

Variables	***r***	***P***
Age	-0,2916	0,0519
Fasting insulin	-0,1752	0,5294
HbA1c	-0,5024	**0,0025****
HOMA-IR	-0,2489	0,3495
CRP	-0,4012	0,0642
Sedimentation	-0,01583	0,9401
Creatinine	0,2517	0,1647
ALT	0,1868	0,2979
AST	0,1435	0,4411
HDL	0,2401	0,1784
LDL	-0,3696	**0,0343***
TC	-0,2177	0,2236
TG	-0,3886	**0,0231***
BUN	-0,03299	0,8841
TSH	-0,1118	0,5101

HgA1c, glycosylated hemoglobin; HOMA-IR, insulin resistance test; CRP, C-Reactive protein; HDL, high density lipoprotein; LDL, low density protein; TG, triglyceride; TC, cholesterol; TSH, thyroid stimulating hormone; BUN, blood urea nitrogen; ALT, alain aminotransferase; AST, aspartate aminotransferase, *P<0.1;**P<0.01; ***P<.0.001; r= Spearman correlation coefficient.

Liu et al. (2016) evaluated plasma netrin-1 levels in newly diagnosed type 2 diabetes patients (n=30) and healthy controls (n=26). It was hereby determined that netrin-1 levels were significantly lower in patients with type 2 diabetes compared to the control group. This evidence was correlated with the results of our study. In this study, we reported that netrin-1 level was negatively correlated with HbA1c, HOMA-IR and fasting blood glucose levels. Although, the average level of HbA1c was recorded higher in obese individuals. In another study, it was reported that serum netrin-1 levels were significantly lower in the prediabetic group than in the control group. In that study, it was determined a negative correlation between serum netrin-1 levels and age, fasting blood glucose, HbA1c, CRP, and sedimentation data (Aydin Acar et al., 2021). Similarly, Nedeva et al. (2020) reported that serum netrin-1 levels were found to be significantly lower in obese, prediabetes and diabetes patient groups compared to the control group. In that study, a negative correlation was reported between BMI and serum netrin-1 levels. Briefly, netrin-1 was slightly increased in individuals with prediabetes. Therefore, it was shown that the netrin-1 levels could be affected by the amount of visceral adipose tissue mass. In correlation with HbA1c, the previous results are in accordance with each other. In this study, it has been suggested that netrin-1 may be involved in the formation of adipose tissue. In parallel with our findings, it was determined that the netrin-1 concentrations decreased in obese individual serum samples and

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst

increased while the BMI ratio was decreased (Nedeva et al., 2020).

Yim et al. (2018) compared serum netrin-1 levels between individuals diagnosed with type 2 diabetes or impaired blood glucose and a control group, and reported that the serum netrin-1 levels were significantly increased in patients group. Also, determined a positive correlation between serum netrin-1 level and HbA1c, fasting blood glucose, and HOMA-IR. Another recent study showed the elevated netrin-1 level in the urine were correlated significantly with insulin resistance in obese individuals diagnosed with kidney failure. A positive correlation of fasting insulin levels and HOMA-IR values with netrin-1 concentrations was also reported. As a result, it was stated that netrin-1 levels in the urine samples can be used as a biomarker for renal failure and insulin resistance in obese children (Ovunc Hamdioglu et al., 2016). In the previous study that carried out with diabetic individuals diagnosed with microalbuminuria, determined significant elevated netrin-1 expressions. Moreover, a positive correlation was found between netrin-1 and HbA1c values (Ay et al., 2016). The link between these two parameters needs to be further clarified, whether netrin-1 can be used as a biomarker for obesity. In line with our preliminary findings, this study hypothesizes that serum netrin-1 level can be used as a potential biomarker in the development of obesity.

In our study, the serum netrin-1 level was determined to be precisely lower in the obese group compared to the healthy control group. In addition, serum netrin-1 level was determined to be negatively correlated with HbA1c, LDL, TG. ROC analysis was performed to measure of the diagnostic ability, clinical sensitivity and specificity of the netrin-1 test to discriminate the two different groups. The AUC was calculated as 0.8667(95% Cl=0.7249-1,000; P<0.0001). An AUC of less than 0.5 is considered indiscriminate, with 0.5-0.6 bad, 0.7-0.8 good, 0.8-0.9 very good, and 0.9-1.0 defined as perfect. Since the range of this study was between 0.8 and 0.9, the serum netrin-1 ELISA method can be considered as a "very good" method in differentiating obese individuals from normal individuals. These results show that netrin-1 can be used as a biomarker in obesity. In our study, a threshold value of <624.2 pg/mL for serum netrin-1 was determined with 80% sensitivity and 93.3% specificity in detecting obesity.

In conclusion, our findings suggest that lower netrin-1 level is associated with obesity and may be a potential biomarker for obesity. However, it needs to be supported by further studies performed with different and larger groups. Meanwhile, we propose with our findings that netrin-1 will be evaluable in the creation of new therapeutic approaches for obesity in the future.

Acknowledgements

This work was supported by the Burdur Mehmet Akif Ersoy University Scientific Research Rrojects Unit [Project number: 0648-YL-20].

Conflict of interest

The authors declare that they have no conflicts of interest.

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

MAKU J. Health Sci. Inst. 2022, 10(2): 116-122.
doi: 10.24998/maeusabed.1094449

Mehmet Akif Ersoy University
Journal of Health Sciences Institute
http://dergipark.gov.tr/maeusabed

Research Article / Araştırma Makalesi

Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions

Batı Akdeniz Bölgesinde Tespit Edilen Bovine Parainfluenza Virus 3 (BPIV3) İzolatının Referenz Suş ve Türkiye İzolatları ile Aminoasit ve Nükleotit Pozisyonlarının Karşılaştırılması

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Abstract: BPIV 3, one of the major viral pathogens of respiratory system disease complex in cattle, is a viral agent generally appearing during autumn and winter months in Northern Hemisphere and causing upper respiratory tract infections. Even though the isolates of this virus are in close antigenic relations, it is classified within three different genotypes as BPIV3 Genotype A, BPIV3 Genotype B and BPIV3 Genotype C. In this research, we aimed to compare the strains of BPIV 3 strain circulating around Western Mediterranean region of Turkey and isolated from different regions of this country and the reference strain of BPIV 3, Shipping Fever (SF-4) with amino acid and nucleotide positions. In the study, previously detected BUR/BPIV 3 isolate with M gene region partially analyzed was used. Phylogenetic researches carried out partially on M gene region in Turkey, different BPIV 3 isolates recorded in gene bank and amino acid and nucleotide positions of BPIV 3 strain detected by ourselves were compared. The changes in Turkey strains of BPIV 3 and nucleotide and amino acid positions of the reference strain were revealed. As a result, detecting base and codon differentiations caused by point mutations among BPIV 3 isolates and correspondingly the appearing amino acid changes was considered crucial in terms of revealing the immunization power of the strain to be used in vaccine production and providing the standardization of BPIV 3 molecular detection.

Keywords: Cattle, Bovine ParainfluenzaVirus3, Amino acids and Nucleotide Positions.

Öz: Sığırlarda solunum sistemi hastalıkları kompleksinin major viral patojenlerden biri olan Bovine Parainfluenza Virus 3 (BPIV3) Kuzey Yarımküre'de genellikle sonbahar ve kış aylarında ortaya çıkan ve üst solunum yolu enfeksiyonlarına yol açan viral bir etkendir. Bu virusun izolatları yakın antijenik ilişki içerisinde olsalar da BPIV3GenotypeA, BPIV3GenotypeB ve BPIV3GenotypeC olarak üç farklı genotipte sınıflandırılmıştır. Bu araştırmada Batı Akdeniz bölgesinde sirkülasyon halinde olan BPIV3 suşunun Türkiye'nin farklı bölgelerinden izole edilmiş suşlar ve BPIV3'ün referenz suşu Shipping Fever (SF-4) ile aminoasit ve nükleotit pozisyonlarının karşılaştırılması amaçlanmıştır. Çalışmada daha önceki araştırmalarımızda tespit ettiğimiz ve M gen bölgesi parsiyel olarak analiz edilmiş BUR/BPIV3 izolatı kullanıldı. SF-4 ile Türkiye'de parsiyel olarak M gen bölgesi üzerine filogenetik araştırmaları ve Genbank'a kayıtları yapılmış farklı BPIV3 izolatları ve bizim tespit ettiğimiz BPIV3 suşunun aminoasit ve nükleotit pozisyonları karşılaştırıldı. BPIV3'ün Türkiye suşları ve referenz suşun nükleotit ve aminoasit pozisyonlarındaki değişimleri ortaya konuldu. Sonuç olarak BPIV3 izolatları arasında noktasal mutasyonlarla meydana gelen baz ve kodon farklılaşmalarının buna bağlı olarak da ortaya çıkan aminoasit değişimlerinin belirlenmesinin aşı üretiminde kullanılacak suşun immunizasyon gücünün ortaya koyulması ve BPIV3'ün moleküler tespitinin standardizasyonunun sağlanması açısından önemli olduğu kanaatine varıldı.

Anahtar Kelimeler: Sığır, Bovine Parainfluenza Virus 3, Aminoasit ve Nükleotit Pozisyonları.

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Geliş tarihi / Received : 28.03.2022 Kabul tarihi / Accepted: 13.06.2022

Introduction

Bovine Respiratory Disease Complex (BRDC) is one of the multi factorial health problems commonly seen around the world. In its etiology, many pathogens can be found such as BPIV3, bovine herpes virus-1 (BHV-1), bovine corona virus (BCoV), bovine respiratory syncytial virus

To cite this article: KÜÇÜK A, YILDIRIM Y. (2022). Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions. MAKU J. Health Sci. Inst., 10(2), 116-122.
ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

(BRSV), bovine adenovirus (BAV), bovine viral diarrhea virus (BVDV) as viral and *Pasteurella multicidae, Mannheimia haemolytica, Histophilus somni* as bacterial (Ellis, 2010). Factors such as weight loss depending on decrease in animal welfare due to infections, decrease in carcass quality, increase in veterinary expenses and prophylaxis, decrease in fertility and animal deaths cause breeders to undergo full scale economical losses (Ellis, 2010).

BPIV 3, one of the most important factors creating BRD complex, is found within *Respirovirus* genus in *Paramyxoviridae* family *Orthoparamyxovirinae* subfamily. The virus has linear, non-segmental (-) ss RNA (Rima et al., 2019). It has three genotypes as BPIV3 genotype A, BPIV3 genotype B and BPIV3 genotype C (Spilki, 2016). BPIV isolates might show genetic differences among themselves. During sequence studies, all genomic areas, except P gene region, have been shown to be protected at high levels (Ellis, 2010). The agent with a pleomorphic morphology usually has an icosahedral structure and is surrounded by a lipid-bilayer membrane (Chamber and Takimoto 2011; Maclachlan et al., 2017). The sequence of BPIV 3 genome with a length of 15.4 kb is as N-P-M-F-HN-L respectively towards 3' 5' and it includes 6 gene regions coding 9 proteins (Ellis, 2010). Out of these 9 proteins, N-M-P-F-HN- and L proteins are structural while V-C and D proteins are non-structural and are synthesized from P gene region (Karron and Collins, 2007; Ellis, 2010). M protein is known to play an important role on the morphology of the virus and infection phase (Elenkumaran, 2013).

The agent is primarily transmitted by droplet and nasal secretion. BPIV 3 that enters the respiratory tract initiates the cellular infectivity by being adsorbed into sialic acid receptors located on cell surfaces (Ellis, 2010). The virus causes viral/bacterial secondary or co infections by creating local immune suppressions in bronchus and bronchiole epithelium cells (Ellis, 2010; Arslan and Küllük, 2017). In field infections, the most commonly isolated viral agents together with BPIV 3 are BHV-1 and BRSV while the bacterial ones are *Mannheimia hemolytica* and *Mycoplasma spp.* (Ellis, 2010; Tiwari et al., 2016). During the studies carried out since the detection of the virus, it has been accepted as an endemic respiratory system infection in cattle populations in each region (Spilki, 2016). The infection is mostly seen during winter and autumn months in Northern Hemisphere (Ellis, 2010). BPIV 3 that might cause infections for cattle of all ages is observed more frequently in animals of 2-8 months old generally. The infections caused by the agent itself alone usually progress sub-clinically while dyspnea, cough, high fever and nasal-conjunctival defluxion occurs if other pathogen agents also participate (Yıldırım, 2009; Ellis, 2010).

In this study, we aimed to compare BPIV 3 strain we isolated in our previous studies (Accession no: MT949524) (Küçük and Yıldırım, 2022), BPIV 3 strains identified based on M gene region in Turkey and amino acid and nucleotide positions and differences of SF-4 strain. Genetic studies among different BPIV3 isolates indicate that regions except the P gene region have a high level of genomic conservation (Ellis 2010). On the other hand, Horwood et al. (2008) revealed different genotypes of the virus as a result of partial analysis of the M gene region of BPIV3. The M gene region was preferred in our study because of its high genomic conservation and its use in genetic typing.

Materials and Methods

Ethics Statement

This research was conducted after the approval of Animal Testing Local Ethics Council (Approvel Number: HADYEK 318/2017).

Virus

In our study we carried out in field research around Burdur, we used BPIV 3 strain that we isolated. This strain was isolated from the nasal swap sample taken from a 7-month-old male cattle displaying general respiratory system infection symptoms such as high fever, cough, abdominal respiration and bilateral mucopurulent nasal flow for 6 days. The nasal swab sample was centrifuged

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

at 4°C for 20 min and 3000 rpm total RNA extraction from the supernatant was carried out according to Rio et al. (2010) and Sample were treated with the one-step RT-PCR kit (Geneall®HyperScript™ one-step RT-PCR master mix, Korea) and RT-PCR was run under described Maidana et al. (2012). Detection of the virus was performed from this nasal swap sample taken in molecular and antigenic ways and sequence analysis was carried out for genetic characterization.

Nucleotide Sequence

Genetic characterization and comparison of the isolates was performed by using 3963-4273 nucleotide positions of partial M gene region (Mfwd: 5´AGTGATCTAGATGATGATCCA 3´ nt and Mrev: 5´GTTATTGATCCAAT TGCTGT − 3´ nt) (Maidana 2012). The consensus nucleotide sequences were verified using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) (Altschul, 1990). The multiple sequence alignments of the data were performed using the Clustal W algorithm. Nucleotide and amino acid alignments and position detections of BPIV 3 strains used in the study were carried out using MegaX program.

Results

The sequence of the M protein was 97-99 % similar to those of the corresponding regions in the partial genome records of the Turkish local strains and 82 % similar of the reference isolates. On the other hand aminoacid similarity is 99,99-100 % similar with local strain and %99,97 similar with SF-4. Nucleotide and amino acid positions of Burdur and Samsun isolates (Accession No:MH357343) were similar. However, 6 nucleotide positions of our isolate and BPIV 3 strain isolated in Erzurum (Accession No:KY511410) were found different. In addition, the aspartic acid (Asp) observed at 1324th position of our isolate transformed into tyrosine (Tyr) in Erzurum isolate. On the other hand, base differences were seen at 45 positions between SF-4 isolate (Accession No:AF178655) that is considered as the reference strain of BPIV 3 and Burdur and Samsun strains. Besides, arginine (Arg) and threonine (Thr) structures located in 1343rd and 1356th amino acid positions of SF-4 respectively transformed into lysine (Lys) and isoleucine (Leu). Amino acid and nucleotide differences detected between the isolates have been shown in Table 1 and Table 2.

Table 1. Comparison of amino acids according to M gene regions of three selected local (Turkey) strains and SF-4.

	Position of Aminoacids		
Isolates	**1 1 2 4**	**1 3 4 3**	**1 3 5 6**
MH357343	Asp	Lys	Ile
KY511410	Tyr	Lys	Ile
MT949524	Asp	Lys	Ile
AF178655	Asp	Arg	Thr

Discussion

BPIV 3 is among the most important factors that provide a basis for respiratory system disease complex (Karron and Colins, 2007). Commonly seen all around the world, the virus affects young herds even though it causes diseases in animals of all races and ages (Fulton, 2010). Serological, virological and molecular researches carried out in different ways revealed that BPIV 3 might also be seen in various farm animals such as sheep, goats, buffaloes and camels as well as cattle (Elenkumaran, 2013). The disease usually occurs during autumn and winter months, progresses subclinically, but causes fatal broncho pneumonias to appear when other factors participate in the infection (Ellis, 2010). Factors such as decrease in carcass quality depending on the infection, prophylaxis, increase in veterinary service expenses and animal deaths cause economical losses for breeders (Fulton, 2010).

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Table 2. Comparison of nucleotides according to M gene regions of three selected local (Turkey) strains and SF-4.

Position of Nucleotides

Isolates	3969	3970	3987	3999	3993	3999	4008	4009	4011	4021	4023	4026	4028	4038	4059	4062	4067	4068	4072	4075	4077	4086	4098	4101
MH357343	G	G	T	A	T	T	T	C	A	C	A	A	A	G	A	C	T	T	T	A	C	G	C	A
KY511410	A	T	T	A	T	T	T	C	A	C	A	A	A	G	A	C	T	T	T	A	C	G	C	A
MT949524	G	G	T	A	T	T	T	C	A	C	A	A	A	G	A	C	T	T	T	A	C	G	C	A
AF178655	A	G	C	G	C	C	A	T	G	T	G	T	G	A	G	T	C	C	C	C	T	A	A	G

Isolates	4104	4107	4119	4125	4128	4131	4131	4134	4137	4149	4152	4161	4164	4170	4194	4200	4206	4212	4218	4230	4251	4254	4266	4269
MH357343	C	A	C	C	G	G	G	C	T	G	C	A	C	A	C	A	G	A	G	C	A	A	T	T
KY511410	C	G	C	C	A	G	G	T	T	G	C	A	C	G	C	A	G	A	G	C	A	A	T	T
MT949524	C	A	C	C	G	G	G	C	T	G	C	A	C	A	C	A	G	A	G	C	A	A	T	T
AF178655	T	G	T	T	G	A	A	C	C	A	T	C	T	A	T	G	A	T	A	A	G	G	C	C

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Limited amount of research is available on phylogenetic analysis of BPIV 3 isolates detected in Turkey. The first phylogenetic analysis of BPIV 3 was performed by Timurkan et al. (2019) (BPIV3/TR/Erz/2014). Soon after this study, Albayrak et al. (2019) carried out the phylogenetic analysis of the strain they isolated in Samsun (Bovine respirovirus 3 isolates Turkey_S1). When viruses containing RNA genomes are compared with DNA based life forms, they display a much higher rate of mutation (Murphy et al., 1999; Lauring et al., 2013). The research conducted showed that viruses with RNA nucleic acid replicated with 10^{-4} -10^{-6} error rate per nucleotide and this number corresponded to a single nucleotide error in almost each cycle (San Juan et al., 2010).

As a result of our study, no difference was seen between nucleotide and amino acid positions of our isolate and BPIV 3 strain detected in Samsun while a difference in 6 nucleotide and in one amino acid position was found between ours and BPIV 3 isolate detected in Erzurum. On the other hand, 45 nucleotide and two amino acid positions were found different between our isolate and BPIV 3 reference strain SF-4. This genetic change between isolates was believed to have been caused by high mutation rates and recombinations occurring in viruses that carry RNA genome.

On the other hand, BPIV3 isolates identified in different geographical regions of Turkey show high phylogenetic similarity. The reason for this is thought to be due to the lack of prophylaxis and biosafety practices, the high prevalence of infection due to the asymptomatic character of the infection, and the dispersal of infected animals to interregional, especially with increased animal movements in some periods.

Fulton et al. (2017) demonstrated the genetic characterization of strains used in the production of BPIV3 modified live vaccines (MLV) and their serological and antigenic relationships with different BPIV3 genotypes that was determined that serum samples from cattle vaccination with MLV vaccines produced with strains in BPIV3genotypeA indicated low antibody levels against BPIV3genotypeC strains that are frequently encountered in the field. Ren et al. (2015) produced six monoclonal antibodies specific for the nucleocapsid (NP) protein of the local strain SD0835 (BPIV3 genotype C) isolate and identified three different antigenic epitopes on the NP using these antibodies. Some monoclonal antibodies were found to be reactive for the BPIV3genotype A and BPIV3genotype C epitopes, while inactive in BPIV3genotype B. In this study, the effect of antigenic variations on immunogenicity among BPIV3 genotypes was revealed**.** Muftuoğlu ve ark. (2021) In the genotype-specific serological study, they carried out in different geographical regions and animal species in Turkey, they found that the BPIV3genotypeC antibody titer was higher than the BPIV3GenotypeA titer all serum samples. They considered the reason for this as a dynamic increase in the prevalence of local virus strains because of geographic isolation and commercial vaccines prepared with BPIV3GenotypeA strain could not provide adequate cross immunization in other BPIV3 genotypes.

In line with this information, it was concluded that genetic differentiation between BPIV 3 strains isolated in different parts of the world may affect vaccine efficacy and standardization of molecular diagnosis of the virus. In addition, it was considered necessary to increase seroprevalence researches on the immune response of reference strains used in vaccine production against isolates in different regions and vaccine development applications using local BPIV 3 isolates might create a more effective immune response.

Conclusion

In this study, BPIV 3 isolate that we detected in a molecular research in Burdur and amino acid and nucleotide changes of the genomic region located between 3963rd-4273rd positions of SF-4,

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considered as the reference strain of BPIV 3, on M gene regions and other BPIV 3 strains detected in Turkey were compared. Our isolate was found different from these strains when we compared nucleotide and amino acid positions of BPIV 3 strains detected in Turkey and the reference BPIV 3 strain, SF-4.

Even though many studies were carried out on prevalence and seroprevalence of BPIV 3 in Turkey, those performed on its phylogenetic are limited. That's why more molecular and phylogenetic research including different gene regions need to be performed to detect circulating BPIV 3 strains in Turkey and to analyze genetic relations. It is thought that molecular and phylogenetic studies to detect local strains of BPIV3 and reveal their genetic differences with other strains will lay the groundwork for genotype-specific vaccine production or diagnostic techniques that may be needed in the future.

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MAKU J. Health Sci. Inst. 2022, 10(2): 123-133.
doi: 10.24998/maeusabed.1136994

Mehmet Akif Ersoy University
Journal of Health Sciences Institute
http://dergipark.gov.tr/maeusabed

Research Article / Araştırma Makalesi

Nephroprotective Effect of Resveratrol Against Methotrexate-Induced Renal Toxicity in Female Rats

Dişi Sıçanlarda Resveratrol'ün Metotreksat ile İndüklenen Renal Toksisiteye Karşı Nefroprotektif Etkisi

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Abstract: The study purposed to appraise the nephroprotective effects of resveratrol-(RES) in relation to methotrexate-(MTX)-induced renal toxicity in female rats. The animals were allocated into three groups with six in each group: control, MTX:(15 mg/kg, only a dose, i.p), MTX+RES group: (15 mg/kg MTX, only a dose, i.p + 20 mg/kg RES, only a dose daily, oral gavage, 7 days). The nephroprotective efficacy was interpreted by measuring biochemical parameters such as serum renal function markers (uric acid, BUN and creatinine), total oxidant (TOS) and antioxidant status (TAS) in renal homogenates. Moreover, the effect of RES on kidneys was appraised by histopathological and immunohistochemical analyzes. In MTX-induced rats, RES treatment exhibited its nephroprotective effects with a significant increase in renal TAS as well as a significant decrease in serum BUN and renal TOS levels. In parallel with the biochemical data, it was observed that RES had a protective effect in the histological staining findings. Immunohistochemically, it was determined that TNF-α, one of the indicators of systemic inflammatory response, decreased with RES-treatment. The findings of the study show that RES administration 1 hour before MTX injection to rats has a curative effect on renal damage.

Keywords: Female rats, Methotrexate, Renal toxicity, Resveratrol.

Öz: Reaktif oksijen türlerinin (ROS) güçlü bir temizleyicisi olan resveratrolün (RES), böbrek hastalıkları da dahil olmak üzere çeşitli metabolik bozukluklara karşı koruyucu etkisi olduğu bildirilmektedir. Bu çalışma, dişi sıçanlarda metotreksat (MTX) ile indüklenen renal toksisitede resveratrolün nefroprotektif etkilerini değerlendirmeyi amaçlamaktadır. Çalışma, her grupta altı adet rat olacak şekilde üç gruba ayrıldı: Kontrol, MTX: (15 mg/kg, tek doz, i.p), MTX + RES grubu: (15 mg/kg MTX, tek doz, i.p + 20 mg/ kg RES, günde tek doz, oral gavaj, 7 gün). Serum renal fonksiyon belirteçleri (ürik asit, BUN ve kreatinin), renal homojenatlarda toplam oksidan (TOS) ve antioksidan durumu (TAS) gibi biyokimyasal parametreler ölçülerek nefroprotektif etkinlik yorumlandı. Ayrıca RES'in böbrekler üzerindeki etkisi histopatolojik ve immünohistokimyasal analizlerle değerlendirildi. MTX ile indüklenen ratlarda RES tedavisi, serum BUN, kreatinin ve renal TOS düzeylerinde anlamlı azalmanın yanında renal TAS'ta anlamlı bir artışla nefro-koruyucu etkilerini gösterdi. Histolojik boyama bulgularında da biyokimyasal verilere paralel olarak, RES'in koruyucu etkisinin olduğu gözlendi. İmmünhistokimyasal olarak sistemik inflamatuar yanıtın göstergelerinden biri olan TNF-α'nın RES tedavisi ile azaldığı belirlendi. Araştırmanın bulguları, ratlara MTX enjeksiyonundan 1 saat önce RES uygulamasının böbrek hasarı üzerinde iyileştirici etkisi olduğunu göstermektedir.

Anahtar Kelimeler: Böbrek toksisitesi, Dişi rat, Metotreksat, Resveratrol.

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Geliş tarihi / Received : 28.06.2022 Kabul tarihi / Accepted: 17.08.2022

To cite this article: YEŞİLOT Ş, ÖZGÖÇMEM M. (2022). Nephroprotective Effect of Resveratrol Against Methotrexate-Induced Renal Toxicity in Female Rats. MAKU J. Health Sci. Inst., 10(2), 123-133.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Introduction

The kidneys perform a significant role in maintaining homeostasis, metabolism and excretion of toxins and drugs/drug metabolites (Perazella, 2009). Therefore, it is the most important organ in terms of drug toxicity. Excessive consumption of a wide variety of therapeutic agents, including antibiotics, nonsteroidal anti-inflammatory drugs, and anticancer agents, causes kidney damage and failure through tubular and glomerular damage (Elmansy et al., 2021). Drug-induced nephrotoxicity depends on their molecular properties, metabolites, and tendency to crystallize and precipitate in tubular lumens (Kwiatkowska et al., 2021).

Methotrexate (4-amino-10-methylfolic acid, MTX), a folic acid antagonist, blocks purine and pyrimidine synthesis by inhibiting several key enzymes, which is responsible for some toxicities as well as its efficacy in cancer therapy (Kremer, 2004; Braun and Rau, 2009). MTX is properly utilized in the treatment of various malignant and non-malignant diseases such as neoplastic diseases, psoriasis, rheumatoid arthritis and lupus erythematosus (Chan and Cronstein, 2013; Bedoui et al., 2019). The adverse effects of MTX often limit its therapeutic applications (Khan et al., 2012; Shah et al., 2016). MTX increases the formation of reactive oxygen species (ROS) and pro-inflammatory cytokines through various mechanisms (Abdel-Raheem and Khedr, 2014; Ju et al., 2020; Kaundal et al., 2021; Hobl et al., 2011). Based on MTX-induced nephrotoxicity studies, renal damage is thought to occur either through precipitation of MTX and its metabolites or through the direct toxic effect of MTX on the renal tubules (Widemann and Adamson, 2006). MTX-induced nephrotoxicity can be reduced by the use of ingredients with anti-oxidant and anti-inflammatory potential (Abouelela et al., 2020; Drishya et al., 2022).

Resveratrol (trans-3,4',5-trihydroxystilbene; (RES)), a polyphenolic compound and natural non-flavonoid antioxidant, is a phytoalexin produced in response to stress in certain plants such as grapes, peanuts, and cranberries (Fremont 2000; Yu et al., 2002). Studies have found that RES is well tolerated at therapeutic doses up to 5 g/day, by evaluating safety and potential mechanisms of activity following multiple dose administration (Nunes et al., 2009; Brown et al., 2010; Calamini et al., 2010). Published studies have shown that RES as a natural phenolic compound and a phytoestrogen is beneficial in the prevention and treatment of cardiovascular diseases, liver disorders, diabetes, cancer, obesity, pain, inflammation, tissue damage, and neurodegeneration. (Baur and Sinclair, 2006; Yeung et al., 2019). It has been shown in animal models that resveratrol can ameliorate various kidney injuries such as diabetic nephropathy, drug-induced injury, and ischemia-reperfusion injury through its antioxidant effect (Kitada and Koya, 2013; Wang et al., 2017). Different inflammatory molecules, especially Tumor necrosis factor-α (TNF-α), one of the proinflammatory cytokines, play a specific role in the development of nephropathy (Navarro and Mora-Fernández, 2006). This study is intended to biochemically and histopathologically examine the potential protective effects of RES on blood and renal tissue against oxidative damage induced by acute MTX exposure.

Materials and Methods

Experimental protocol

Female Wistar Albino rats (weighing between 240-360 g) were purchased from Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Center, used in the experiment. The animals were maintained in climate-controlled rooms (25 °C; 55% humidity) with diurnal lighting (12:12-h light:dark photoperiod). The rats had access to standard rodent chow and tap water ad libitum throughout the whole study. All animal use and accompanying procedures were in accordance with the animal research guidelines of the National Institutes of Health and were Burdur Mehmet Akif Ersoy University Animal

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Experiments Local Ethics Committee-approved (Ethical approval number: 17.03.2021-742).

In our study, 3 different experimental groups, each consisting of 6 female rats, were formed. Group-1, named as the control group, was treated with a single dose of 0.9% saline (1 mL/kg) intraperitoneal injection (i.p) on the 1st day. Group-2 was given only a single dose of 15 mg/kg i.p MTX (Kocaman and Çolakoğlu, 2013); Group-3 was given RES (Yuluğ et al., 2013) at a dose of 20 mg/kg 1 h before MTX (a single dose of 15 mg/kg i.p) administration by oral gavage. Group-3 was administered RES at the same time for 7 days. 24 hours after the last administration, on the 8th experimental day, the animals were euthanized by surgical anesthesia of 10% ketamine HCl (Ketalar® Eczacıbaşı, İstanbul) and 2% xylazine (Alfazin amp) administered intraperitonealy in groups. Until the end of the experiment, 2 rats from group-3 died, and the data of the study were evaluated accordingly. After anesthesia, blood and kidney tissue samples were taken. The blood samples were centrifuged at 5000 rpm for 10 minutes and serum samples were obtained to analyze the kidney function tests. One of the kidney tissues of each sample was taken to be homogenized for biochemical analysis, while the other kidney was placed in 10% formaldehyde solution for histopathological studies.

Preparation of renal tissue samples

Renal tissues of each group stored at -20 ºC were weighed separately after being brought to room temperature and diluted 10 times with 50 mM phosphate buffer (pH 7.4). The homogenization was completed by treatment with tissue shredder (Janke & Kuntel Ultraturrax T-25, Germany) and then sonicator (UW-2070 Bandeun Electronic, Germany). The samples were centrifuged at 10.000 rpm, 10 min. The renal supernatants were transferred to eppendorf tubes and used in further studies to determine the oxidant/antioxidant status.

Biochemical anaylsis

The biochemical parameters, a sign of kidney function such as uric acid, blood urea nitrogen (BUN) and creatinine (Cr) in serum were measured on an automatic clinical chemistry analyzer (Gesan chem 200, Italy) device in Veterinary Training Hospital of Burdur Mehmet Akif Ersoy University.

Total Oxidants and Antioxidants Status

TOS (Total Oxidant Status) and TAS (Total Antioxidant Status) parameters were studied by spectrophotometric method using Rel Assay Diagnostic kits (Mega Tip, Gaziantep, Turkey) and Biotek® (Epoch 2 Microplate Spectrophotometer) microplate reader in the renal supernatants obtained. TOS results were expressed in μmoL H_2O_2 equivalent/L (μmol H_2O_2 eq/L). TAS results of the samples were clarified as mmol Trolox equivalent/L (mmol Trolox eq/L). Establishing of OSI, which is an determinative parameter of oxidative stress level, the ratio of TOS to TAS was calculated using the following formula:

OSI (arbitrary unit)
= [((TOS, μmol/L))
/ (TAS, μmol Trolox equivalent/L)X 100]

Histopathological procedure

Renal tissues were removed from each rat and after cleaning they were washed in aqua over night. Than tissues were fixed in 10% neutral buffered formalin and dehydrated in 50–100% ethanol, made transparentin xylol. After all tissues buried in paraffin and were cut to 3–5 μ. At last they were stained with hematoxylin and eosin (H-E). The slides were examined using a light microscope (LeicaSM2000R, Germany) and photographed. Degeneration evelations were made acording to method of by Refaiy et al., (2011).

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Immunohistochemical procedure

The samples were stained with TNF-α primary ab (rabbit-anti-TNF-α antibody, Abcam, Cambridge-USA). Semi-quantitative evaluation method was used by Refaiy et al. (2011) to describe the observed staining intensities.

The staining score for H&E and IHC was evaluated as;
(-),0; none staining
(+), 1 mild staining
(++), 2 moderate staining
(+++), 3 intense staining.

Statistical Analysis

Statistical analyzes of the study were made using the IBM SPSS 20.0 program. All results are expressed as mean ± standard error. In histological analysis, Kruskal-Wallis test was used for semi-qualitative evaluation and non-parametric Mann-Whitney U test was used for pairwise comparisons. One-way ANOVA was used for intergroup comparison in biochemical analyses. The valuation of p below 0.05 were considered significant.

Results

Biochemical markers of renal function

The serum levels of uric acid was not significantly affected, but the BUN and Cr values in the MTX-induced female rats demostrated a significant increase in comparison with the control. RES admistration significantly decreased BUN parameter (Table 1), ($p<0.05$).

Table 1. Biochemical parameters in the serum.

	Uric acid (mmol/dL)		BUN (mg/dL)		Creatinine (mg/dL)	
Groups	Mean ±SD	P	Mean ±SD	P	Mean ±SD	P
Control	0.58±0.64		17.13±2.48	**p<0.000	0.27±0.04	
MTX	0.80±0.44		22.11±0.63	*p<0.000	0.33±0.02	*p=0.021
MTX+RES	1.28±0.60		20.09±0.66	*p=0.036 **p=0.008	0.30±0.03	

MTX - Methotrexate; RES - Resveratrol. Values are presented as means±SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. *p: Comparison with the control, **p: Comparison with the MTX.

Table 2. TOS, TAS and OSI values of renal tissues.

	TOS (μmol H_2O_2 eq/L)		TAS (mmol Trolox eq/L)		OSI (AU)	
Groups	Mean ±SD	P	Mean ±SD	P	Mean ±SD	P
Control	43.43±4.10	** p=0.001	0.97±0.04	**p=0.015	4.47±0.40	** p=0.005
MTX	98.35±11.94	* p=0.001	0.77±0.09	*p=0.015	12.94±2.58	*p=0.005
MTX+RES	28.53±0.00	*p=0.004 ** p=0.001	1.17±0.07	*p=0.005 ** p<0.001	2.44±0.15	*p<0.001 **p=0.002

MTX - Methotrexate; RES - Resveratrol. Data are presented as means±SD. One way ANOVA was used for comparison between groups.

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Table 3. Grading histological structural changes according to groups.

	Group 1 Control (n=6)	Group 2 MTX (n= 6)	Group 3 MTX + RES (n= 4)
Glomerules Degeneration and Vacuolization	-	++	+
Tubular Dilatation and Degeneration	-/+	++	+/-
Enlargements in Bowman Capsules	-/+	++	+/-
Mononuclear Cell Infiltration	-	+++	+++/++

Oxidative stress parameters in the renal homogenates

TOS, an indicator of the formation of ROS, increased significantly in group-2 compared to control, but decreased in group-3 (p=0.001 and 0.004, respectively). It was observed that RES therapy significantly decreased the TOS level compare with group-2 (Table 2), (p=0.001). TAS, an indicator of antioxidant capacity, was significantly decreased in group-2 and increased in the group-3 compared to the control (p=0.015, and 0.005, respectively). It was observed that RES therapy significantly increased the TAS level compare with group-2 (Table 2), (p<0.001). It was clearly seen that the OSI increased significantly in the group-2 compared to the control (p=0.005), and decreased significantly in the RES-treated group (p<0.001). It is seen that RES treatment significantly reduces the OSI level when compared to group-2. (p=0.002), (Table 2).

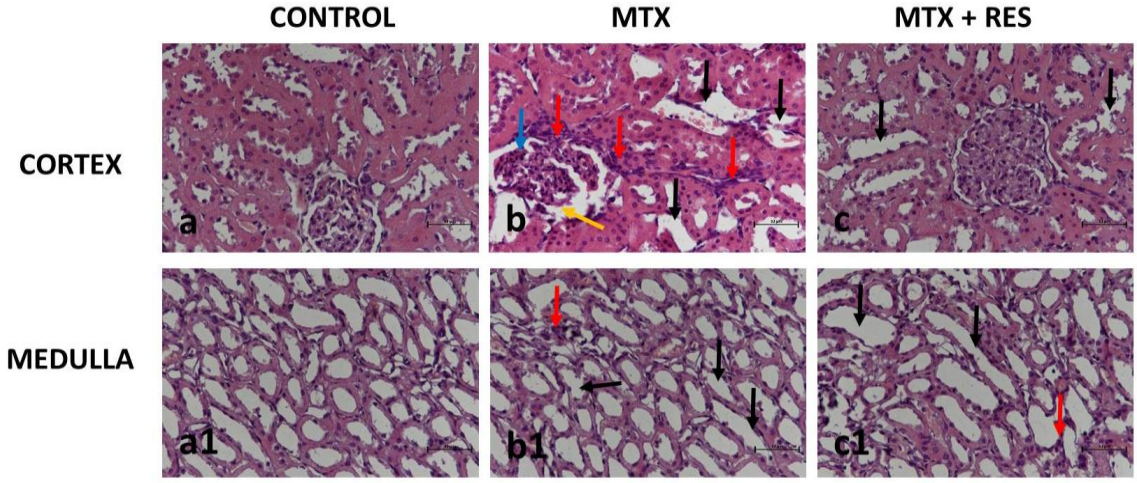

Figure 1. Control group: Kidney tissues were normal in control and there was very little tubular dilatation, too. MTX and MTX+RES group: Glomerules vacuolization (yellow arrow), glomerules degeneration (blue arrow), tubular dilatations and degeneration (black arrow), mononuclear cell infiltrations (red arrow), (a,b,c; kortexs - a1,b1,c1; medulla, H-E x400).

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Histopathological findings of kidney

The findings observed under the light microscope are given in Table 3. Histopathology of renal tissues were normal appearance in group-1, but also there was very little tubular dilatation, too (Table 3, Fig.1; a-a1), ($p>0.05$). The histopathological changes were observed significantly in group-2, which were remarkabled; degeneration of glomerules and vacuolization, tubular degeneration, tubular dilatation in the most of distal and proximal tubules, enlargements in Bowman capsules and mononuclear cell infiltration in the intertubular and perivascular fields (Table 3, Fig.1; b-b1). These histopathological findings were mostly observed in group-2 compared to group-3 (Table 3, Fig.1; c-c1), ($p<0.05$).

Immunohistochemical findings of kidney

A semi-quantitative assessment determined that the renal tissues of rats in group-1 had either very mild or no TNF-α staining. (Table 4, Fig.2; a), ($p<0.05$). However, an intense level of staining was seen in group-2 (Table 4, Fig. 2; b), while it was mild in group-3 (Table 4, Fig. 2; c).

Table 4. TNF-α staining**.**

	Control Group 1 (n=6)	MTX Group 2 (n=6)	MTX+RES Group 3 (n=4)
TNF-α	**-**	+/++	+/-

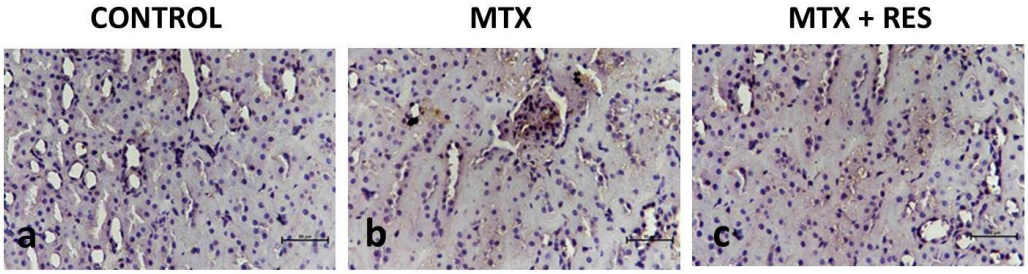

Figure 2. Control group a; staining was either very mild or nonexistent. MTX group b; showed mild/intense staining intensity compared with other groups. Immunohistological sections from MTX+RES group c; showed less staining intensity than group MTX b, (TNF- α immunstaining, ×400).

Discussion

The current study was conducted to evaluate whether RES can prevent or reduce MTX-induced renal injury by examining different biochemical and histopathological parameters related to renal function of female rats. Biochemical and histopathological findings clearly showed significant changes in renal function due to increased renal oxidative stress after MTX exposure. Available data suggest that RES can ameliorate MTX-induced renal damage by altering the levels of endogenous antioxidants.

Since the kidneys are responsible for the biotransformation and elimination of various toxins and drugs, they tend to generate free radicals that are involved in the pathogenesis of renal injury (Singh et al., 2003; Perazella, 2009). MTX administration is routinely applied in the treatment of malignant and non-malignant ailments and various systemic adverse effects are seen (Green and Chamberlain, 2009; Sotoudehmanesh et al., 2010; Gaies et al., 2012). Studies indicate that the administration of MTX produces functional and morphological changes in the kidney due to the direct toxic effects of the

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drug. MTX cytotoxicity and damage to the renal tubules are associated with the formation of free radicals and oxidative stress (Savran et al., 2017; Heidari et al., 2018).

The current study showed that while MTX caused an increment in serum BUN and Cr values, it did not cause a significant increment in uric acid values (Table 1). The findings are in line with the results of the investigation by Asci et al. (2017) reporting that MTX has a major role in the pathogenesis of renal dysfunction. Armağan et al. (2015) also detected biochemical results parallel to our study on MTX-induced renal dysfunction. Increased concentrations of serum urea, BUN, and Cr may be an indication of ROS generation via MTX in the kidney, resulting in kidney damage/deficiency (Abdel-Raheem and Khedr, 2014; Ahmed et al., 2015).

Oxidative stress induces damage to DNA and cellular biomolecules, resulting in degradation of cellular redox homeostasis, cellular apoptosis, and abnormal activation of signaling pathways (Sesti et al., 2012). TOS, which is an indicator of oxidation capacity, increased in support of the oxidative damage caused by MTX admistration. TAS, which is an indicator of antioxidant capacity, increased, indicating that RES teratment creates an antioxidant effect. It has been shown that MTX can modify the activity and levels of some ingredient of the tissue antioxidant defense system, increment the production of free radicals, especially ROS, and the formation of lipid peroxidation (Abdel-Raheem and Khedr, 2014; Armagan et al., 2015; Kandemir et al., 2017; Asci et al., 2017). It is known that while RES is a weak antioxidant in vitro, it is a strong antioxidant in vivo due to nitric oxide synthesis and free radical scavenging effect (Bay Karabulut, 2008). The findings of the study suggest that RES ameliorates the adverse effects of MTX on TAS and TOS. Many previous studies are in line with present findings, as they indicate that RES can directly scavenge reactive oxygen species such as toxic hydroxyl and superoxide radicals in the kidneys (Yu et al., 2013; Zhang et al., 2014; Shahbazi et al., 2020). Although it has been shown in different studies that RES improves renal damage with its antioxidant properties, there are not enough studies on oxidative stress and antioxidant markers TOS, TAS and OSI in MTX-induced nephrotoxicity. TAS and TOS levels were evaluated in MTX-induced organ damage studies performed with antioxidant agents in different tissues (Gunyeli et al., 2021; Soylu Karapınar et al., 2017; Özgöçmen and Yeşilot, 2021). In addition, agents with different antioxidant properties such as vitamin E (Taghizadieh et al., 2014), quercetin (Erboga et al., 2015; Yuksel et al., 2017), silymarin and naringin (Kandemir et al., 2017), gallic acid (Asci et al., 2017) vitamin C (Savran et al., 2017), rutin (Tambağ et al., 2021) have been shown to have protective effects in renal damage caused by MTX.

In the present study, the preventive effect of RES towards MTX-induced oxidative stress-mediated renal disfunction was evaluated at the histopathological level. In the study by El-Sheikh et al., while significant glomerular damage, enlarged Bowman's space, tubular necrosis, leukocytic infiltration and hyaline eruptions and deterioration in kidney structure were observed in the histopathology of the kidney of the MTX-treated rats, normal findings similar to the control group were observed in the RES-treated group (El-Sheikh et al., 2016). Silan et al. showed that RES has a protective effect against gentamicin-induced nephrotoxicity histopathologically, lipid peroxidation and cellular damage. In the histopathology slides of the same study, less parietal cell hyperplasia, tubular vacuolization, and tubular necrosis were detected in resveratrol-treated rats compared to the gentamicin-treated group (Silan et al., 2007). Consistent with our patho-histological results, RES has been previously reported to have nephroprotective efficacy in other models of induced kidney injury in which it was involved (Yu et al., 2013; Akbel et al., 2018; Shahbazi et al., 2020). The authors of the studies concluded that the free radical scavenging property of resveratrol may be

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responsible for its nephroprotective effects against induced nephrotoxicity.

The primary pathological mechanism linking oxidative stress, inflammation, and progression of kidney disease is the initiation of kidney damage due to the inflammatory response resulting from the activities of intracellular and extracellular oxygen-derived radicals (Elmarakby and Sullivan, 2012; Tucker et al., 2015; Ker et al., 2020). Activation of proinflammatory cytokines and generation of inflammatory response are associated with MTX-induced renal toxicity (Çakır et al., 2015). It is known that TNF-α is an important proinflammatory cytokine and a pathogenic factor in renal damage (Baud and Ardaillou, 1995; Navarro and Mora-Fernández, 2006). In the current study, it was detected immunohistochemically that TNF-α, one of the systemic inflammatory response indicators, increased in the MTX group and decreased in the RES treatment group (Table 4, Fig.2). The nephroprotective effects of RES are not limited to amelioration of pathological renal fibrosis, but also include renal morbidities. RES shows protective effects mostly against various renal damage due to its antioxidant properties (Malhotra et al., 2015). The findings of Jang et al show that resveratrol exerts protective effects on aging kidneys by reducing oxidative stress, inflammation and fibrosis through Ang II suppression and MasR activation (Jang et al., 2018). In the study by Kandemir et al., (2017) it was declared that TNF-α expression in kidney tissue increased with MTX application. Studies showing that RES reduces kidney damage through modulation of oxidative stress and TNF-α-induced inflammation in rats are consistent with current study (Saldanha et al., 2013; El-Sheikh et al., 2017; Wang et al., 2020). These results are associated with the nephroprotective antioxidant effects of RES on MTX-induced nephrotoxicity.

Conclusion

The indications suggest that oxidative stress reasoned by aberrant ROS formation is responsible for the pathophysiology of MTX-induced nephrotoxicity. RES treatment ameliorates MTX nephrotoxicity in female rats by restoring kidney functions and inhibiting TNF- α with its free radical scavenging and natural antioxidant effects. As a dietary supplement, RES can be used with MTX therapy as it reduces nephrotoxic side effects. Therefore, RES supplementation as adjuvant therapy may be promising in alleviating the systemic adverse effects of drugs.

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