T.C.
DOKUZ EYLÜL ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ

DEPRESYONDA DNA HASARININ VE ONARIMININ BELİRTİLİ DÖNEM VE DÜZELME İLE İLİŞKİSİ

Dr. DENİZ CEYLAN TUFAN ÖZALP

TEMEL SİNİRBİLİMLER

DOKTORA TEZİ

İZMİR-2019

TEZ KODU: DEU.HSI.PhD-2012970068

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(Bu araştırma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından 216S778 proje numarası ile desteklenmiştir.)

TEZ KODU: DEU.HSI.PhD-2012970068

Dokuz Eylül Üniversitesi Sağlık Bilimleri Enstitüsü Sinirbilim Anabilim Dalı, Temel Sinirbilim Doktora programı öğrencisi Dr. Deniz CEYLAN TUFAN ÖZALP **"DEPRESYONDA DNA HASARININ VE ONARIMININ BELİRTİLİ DÖNEM VE DÜZELME İLE İLİŞKİSİ"** konulu Doktora tezini 06/03/2018 tarihinde başarılı olarak tamamlamıştır.

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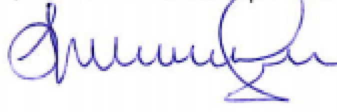

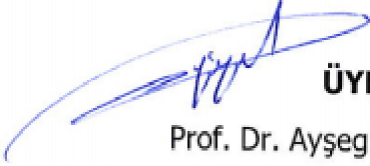

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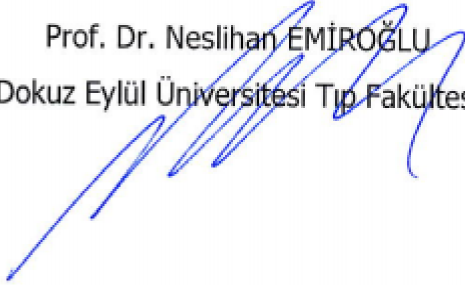

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KISALTMALAR

8**-OHdG:** 8-hidroksideoksiguanozin

BÇO: Baz kesip çıkarma onarımı

DNA: Deoksiribonükleik asit

GZ-PZR: Gerçek zamanlı polimeraz zincir reaksiyonu

LC-MS-MS: Sıvı kromatografi-tandem kütle spektrometresi

OGG-1: 8-oksoguanin DNA glikozilaz 1

TEŞEKKÜR

Sevgili danışmanım, değerli bilim insanı Prof. Dr. Ayşegül Özerdem'e bu tez çalışmasına olduğu kadar yaşamıma kattığı her şey için; bilgisini, sevgisini ve desteğini cömertçe paylaştığı için sonsuz teşekkür ediyorum.

Bilimsel ve etik değerleri her zaman yolumu aydınlatan sevgili hocam Prof. Dr. Zeliha Tunca'ya bir hekim ve araştırmacı olarak yetişmemdeki emeği için ne kadar teşekkür etsem azdır.

Tez izleme komitemin çok değerli üyeleri Prof. Dr. Pınar Akan'a ve Prof. Dr. Şermin Genç'e bu tez projesine ve bilimsel heyecanıma katkıları için, her sorunda araştırma ekipleriyle birlikte yardımıma koştukları için çok teşekkür ediyorum.

Projemizin TÜBİTAK desteği almasına, projenin yürütülmesine katkıları, huzurlu çalışma ortaklıkları için Prof. Dr. Hüray İşlekel'e, Dr. Öğr. Üyesi Gamze Tuna'ya, Dr. Melis Kant'a ve Dr. Merve Akış'a teşekkürlerimi sunuyorum.

Projemizin klinik hasta alımı ve izlem işlemlerindeki destekleri için Ayşegül Ildız'a, Burcu Verim'e ve Ayşe Er'e, projenin tamamlanmasında gösterdiği azimli çalışkanlık için Selda Yılmaz'a ve klinik aşamada emek veren tüm arkadaşlarıma çok teşekkür ediyorum.

İzmir Ekonomi Üniversitesi Rektörü Prof. Dr. Murat Aşkar'a ve Teknoloji Transfer Ofisi'ne projenin yürütülmesine katkıları için, İzmir Ekonomi Üniversitesi Tıp Fakültesi Dekanı Prof. Dr. Hakan Abacıoğlu'na ve Sağlık Hizmetleri Meslek Yüksekokulu Müdürü Prof. Dr. İlgi Şemin'e projemize sundukları destek için teşekkürlerimi sunuyorum.

Projemize verdikleri bütçe destekleri ile işlerimizi kolaylaştıran ve çalışma azmimizi arttıran Türkiye Psikiyatri Derneği'ne ve TÜBİTAK'a teşekkür ediyorum.

Tez çalışmam boyunca gösterdiği sabır ve destek için sevgili eşim Erkin'e sonsuz teşekkür ediyorum.

Son olarak, tüm bilimsel çalışmalarıma ve hayatıma anlam katan sevgili hastalarımıza çok teşekkür ediyorum.

ÖZET

Depresyonda DNA hasarının ve Onarımının Belirtili Dönem ve Düzelme İle İlişkisi

Deniz CEYLAN TUFAN ÖZALP

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Giriş ve amaç: Depresyonda tıbbi eş tanılılık, bilişsel bozukluklar ve mortalite riskinde artış görülmesi, DNA hasarı/onarımı mekanizmalarının depresyonun etyopatogenezinde merkezi bir role sahip olabileceğini düşündürmektedir. Bu çalışmada DNA hasarı/onarımı ile depresif belirtiler, depresif belirtilerin şiddeti ve belirtilerin düzelmesi arasındaki ilişkilerin değerlendirilmesi amaçlanmıştır.

Yöntemler: Araştırmaya 33 unipolar depresyon, 24 bipolar depresyon tanılı hasta ve 61 sağlıklı gönüllü dahil edilmiştir. İlk görüşmede tanısal görüşmeler ve klinik ölçümler yapılmış, katılımcıların kan ve idrar örnekleri alınmıştır. Hastaların klinik ve laboratuvar ölçümleri araştırmaya dahil edildikten 8 hafta sonra tekrar edilmiş, 8. haftadaki klinik ölçümlerde düzelme saptanmayan hastaların son ölçümleri 12. haftada tekrarlanmıştır. Düzelme 17 maddeli Hamilton Depresyon Ölçeği toplam puanının ≤ 7 olması ile tanımlanmıştır. Katılımcılardan elde edilecek idrar örneklerinde hasarlı DNA nükleozidleri Sıvı Kromatografi- Tandem Kütle Spektrometresi kullanılarak ölçülmüştür. İdrar 8-OHdG düzeyleri, idrar kreatinin düzeylerine göre düzeltilmiştir. Kan örneklerinden elde edilen cDNA örneklerinden gerçek zamanlı polimeraz zincir reaksiyonu ile OGG1 mRNA ekspresyon düzeyleri belirlenmiştir.

Bulgular: Depresyon hastalarının idrar 8-OHdG düzeyleri sağlıklı bireylerinkine göre yüksek ($p=0,008$), OGG-1 ekspresyon düzeylerinin sağlıklı bireylere göre düşük olarak saptanmıştır ($p=0,024$). Depresif bireylerin belirtilerinin düzelmesiyle 8-OHdG düzeyleri azalmış ($p=0,001$), OGG-1 ekspresyon düzeyleri 2,95 kat artmış ($p= 0,046$) olarak saptanmıştır.

Sonuç: Araştırmanın sonuçları depresyon hastalarında sağlıklı kontrollere göre daha fazla oksidatif DNA hasarı olduğunu; DNA hasar lezyonlarının DNA onarım sistemindeki geri dönüşümlü bozulmaların sonucu olabileceğini düşündürmektedir. Verilerimiz, DNA hasar/onarım süreçlerinin depresif dönemlerin nörobiyolojisi ile ilişkili olabileceğini göstermektedir.

Anahtar sözcükler: Depresyon, DNA hasarı, DNA onarımı, 8-OHdG, OGG-1

ABSTRACT

The Association of DNA Damage and Repair with Symptomatic and Remitted States of Depression

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Introduction: Presence of higher risk for medical comorbidity, cognitive impairments and mortality in depression suggests a central role of DNA damage/repair mechanisms in etiopathogenesis of depression. The aim of the study was to determine the relationship between DNA damage/repair and the depressive symptoms, symptom severity and symptomatic remission in depression.

Methods: Thirty three unipolar depression, 24 bipolar depression and 61 healthy volunteers were included in the study. In the first visit, diagnostic interviews and clinical assessments were applied and blood and urine samples of participants were obtained. Clinical and laboratory assessments were repeated at the 8th week. The last assessments were performed at the 12th week for patients determined as unremitted by clinical assessments. The remission is defined as 17-items Hamilton Depression Scale score of 7 or lower. Liquid chromatography tandem mass spectrometry was used in the measurements of damaged DNA nucleosides in urine samples. The levels of 8-OHdG were adjusted for urine creatinine levels. The levels of OGG1 expressions were determined from cDNA samples, extracted from blood samples, using real time-polymerase chain reaction.

Results: Patients with depression have higher levels of 8-OHdG (p=0,008), lower levels of OGG-1 (p=0,024) than healthy controls. Patients with depression presented decreased levels of 8-OHdG (p=0,001), 2,95 fold increased levels of OGG-1 by remission of depressive episode.

Conclusion: Our results suggest that patients with depression, compared with healthy volunteers, had more oxidative DNA damage, and that those lesions may be accumulated by reversible impairments of the DNA repair systems. Our data indicate that DNA damage/repair processes might be involved in neurobiology of depressive episodes.

Key words: Depression, DNA damage, DNA repair, 8-OHdG, OGG-1

1. GİRİŞ VE AMAÇ

1.1. Problemin Tanımı ve Önemi

Dünya Sağlık Örgütünün verilerine göre depresyon dünya genelinde halk sağlığını en çok tehdit eden sorunlar arasındadır (1). Depresyonda kardiyovasküler bozukluklar, kanser, obezite, diyabet gibi genel tıbbi durumlara bağlı mortalite ve morbidite arttırmakta, ve önemli tıbbi hastalıkların %20-25'ine de depresyon eşlik etmektedir (2,3). Kişiye ve topluma kısa ve uzun dönemli yüksek maliyeti olan depresyonun altında yatan biyolojik mekanizmalar ise henüz bütünüyle aydınlatılamamıştır. Depresyonda yeni ve tedavilerin geliştirilebilmesi ve tedavi yanıtlarının kanıta dayalı olarak öngörülebilmesi için hastalığın biyolojik mekanizmalarının anlaşılmasına ve depresyon kliniği sırasında bozulan dizgelerin aydınlatılmasına gereksinim vardır. Depresyon hastalarının, tıbbi eş tanılılık, bilişsel bozukluklar ve mortalite açısından yüksek risk taşımakta oluşu, oksidatif stresin, kronik inflamatuar süreçlerin ve hücresel yaşlanmanın depresyonun nörobiyolojisinde merkezi bir role sahip olabileceğini düşündürmektedir (4).

Oksidatif DNA hasarı belirteçleri, depresyon için biyolojik belirteç adayları arasında gösterilmektedir (5). DNA hasarı ve onarımı süreğen ve dinamik bir süreçtir; sağlıklı bireylerde, oksidatif DNA hasarı ve çeşitli onarım sistemleri dengeli biçimde etkileşimdedir (6). DNA hasarı ve onarımı arasındaki dengede bozulma, DNA zincirinin kırılmasına, nükleotid kaybına ve nükleotidlerdeki bazlarda modifikasyonlara yol açar ve mutagenezin, karsinogenezin, çeşitli hastalıkların ve yaşlanmanın etyopatogenezine katılır (6). Güncel bir metaanaliz çalışmasında, oksidatif DNA hasarı belirteci olan 8-hidroksideoksiguanozin (8-OHdG) düzeylerinin depresyonda artmış olduğunu gösterilmiş, bu konuda yeni araştırmalara gereksinim olduğu belirtilmiştir (7). Araştırma grubumuzun bipolar bozuklukta DNA hasarı üzerine yapmış olduğu çalışmalar, bipolar bozuklukta mani ve depresyon dönemlerinde DNA hasarında artış saptandığını göstermiştir (8). Bu bulgularımız, depresyonda DNA hasarı/onarımı üzerine daha ayrıntılı araştırmalara gereksinim olduğunu düşündürmektedir.

Oksidasyona uğrayan DNA onarıma girdiği için, DNA lezyonlarının onarım ürünleri (örneğin okside nükleozidler ve bazlar), metabolize olmadan idrarla atılır. İdrar girişimsel olmayan ve kolay elde edilen bir numune olduğu için, oksidatif DNA hasarı ölçümünde idrarda 8-OHdG sık kullanılan bir belirteçtir (9). Depresyonda DNA hasarını inceleyen güncel araştırmalar DNA oksidasyon belirteci olarak idrar 8-OHdG düzeylerini kullanmaktadır (10-13). Psikiyatrik hastalıklarda DNA onarımı ile ilgili bilgiler çok sınırlıdır. Kanser hastalarında,

depresyon eş tanısı olanlar hastalarda hasarlı guanin bazlarını kesip çıkarmak için özelleşmiş temel bir enzim olan, insan 8-oksoguanin DNA glikozilazın (8-oxoguanine DNA glycosylase-1; OGG-1) ekspresyonun depresyon eş tanısı olmayanlardan daha yüksek olduğu gösterilmiştir (14,15). Araştırma grubumuz bipolar bozukluğun ötimi döneminde OGG-1 ekspresyonunun sağlıklı bireylere göre azalmış olduğunu saptamıştır (16). Bu bulgumuzla uyumlu olarak güncel bir araştırmada hızlı döngülü bipolar bozuklukta OGG-1 ekspresyonunda azalma bildirilmiştir (17).

Depresyonda belirtilerin varlığı ve düzelmesiyle DNA hasar/onarım profili arasındaki ilişkiyi inceleyen ileriye dönük desende bir izleme çalışması bulunmamaktadır. Literatürdeki çalışmalar, yaşam stili, beslenme alışkanlıkları, sosyoekonomik koşullar gibi çeşitli faktörleri karıştırıcı etkisini azaltabilecek bir desene de sahip değildir. DNA onarımı mekanizmalarının depresyon kliniği bileşenleri ile ilişkisini inceleyen bir araştırma bulunmamaktadır. Araştırmaların büyük kısmında DNA hasarı ölçümünde kromatografik yöntemler kullanılmamıştır (6,7). Bu araştırmada depresyon seyrinde oksidatif DNA hasarının ve onarımının ne derece rolünün olduğu sorgulanmaktadır.

1.2. Araştırmanın Amacı ve Hipotezleri

Bu çalışmada DNA hasarı/onarımı ile depresif belirtiler, depresif belirtilerin şiddeti ve belirtilerin düzelmesi arasındaki ilişkilerin değerlendirilmesi amaçlanmıştır. Araştırmanın hipotezleri şunlardır:

H1: Depresif hastaların idrar 8-OHdG düzeylerinin ortalaması sağlıklı gönüllülerinkinden yüksektir.

H2: Depresif hastaların OGG-1 mRNA ekspresyon düzeyleri ortalaması sağlıklı gönüllülerinkinden farklıdır.

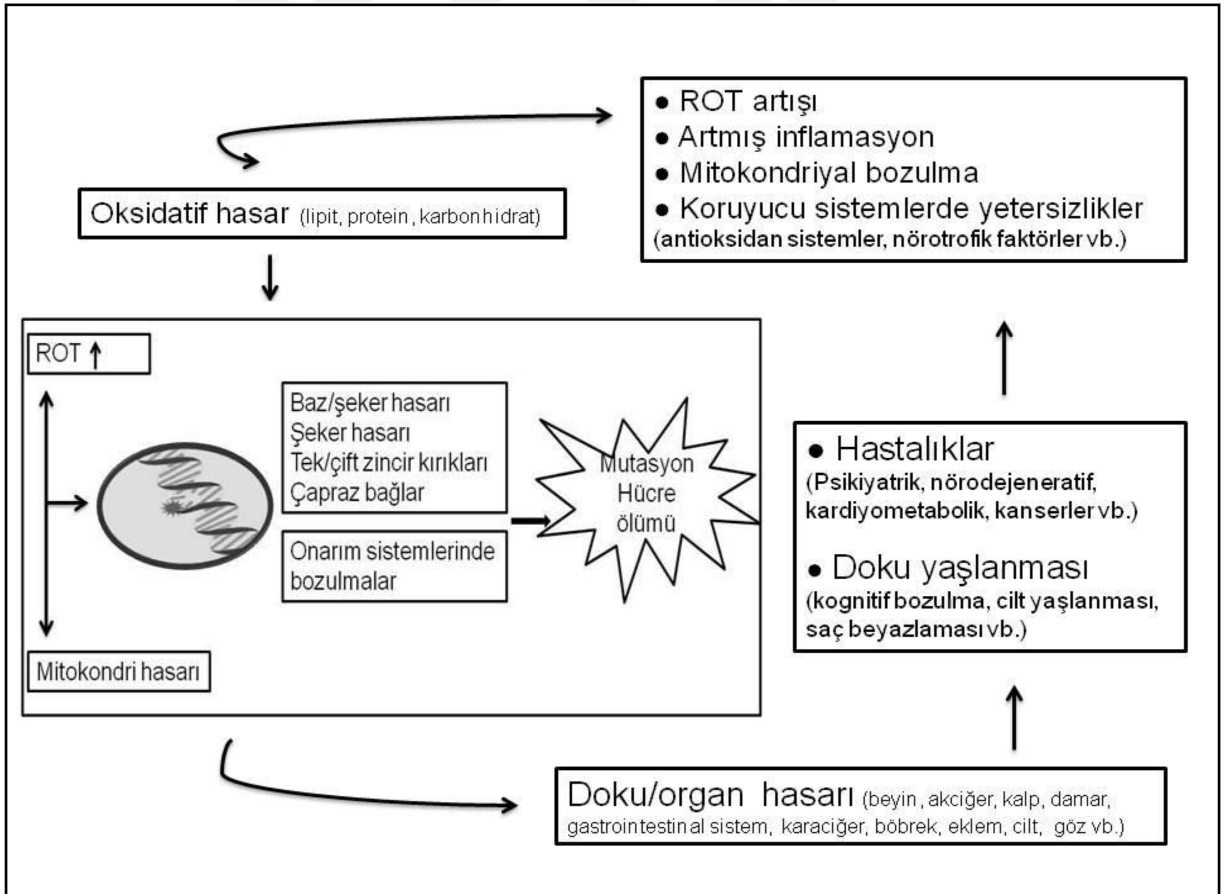
H3: Depresif belirtilerin düzelmesi sonrası 8-OHdG ve OGG-1 mRNA ekspresyonu düzeyleri depresif belirtilerin olduğu dönemdekilere göre farklılıklar gösterir.

2. GENEL BİLGİLER

Son yıllarda depresif bozukluklarda oksidatif stresin rolü üzerine yeni kanıtlar ortaya çıkmaktadır. Depresyonda oksidatif stres belirteçlerinde artış ve antioksidan savunmada azalma olduğu meta-analitik yöntem kullanılarak gösterilmiştir (18). Depresyonda oksidatif DNA hasarının ve onarımının incelenmesi ise güncel konu başlıklarıdır.

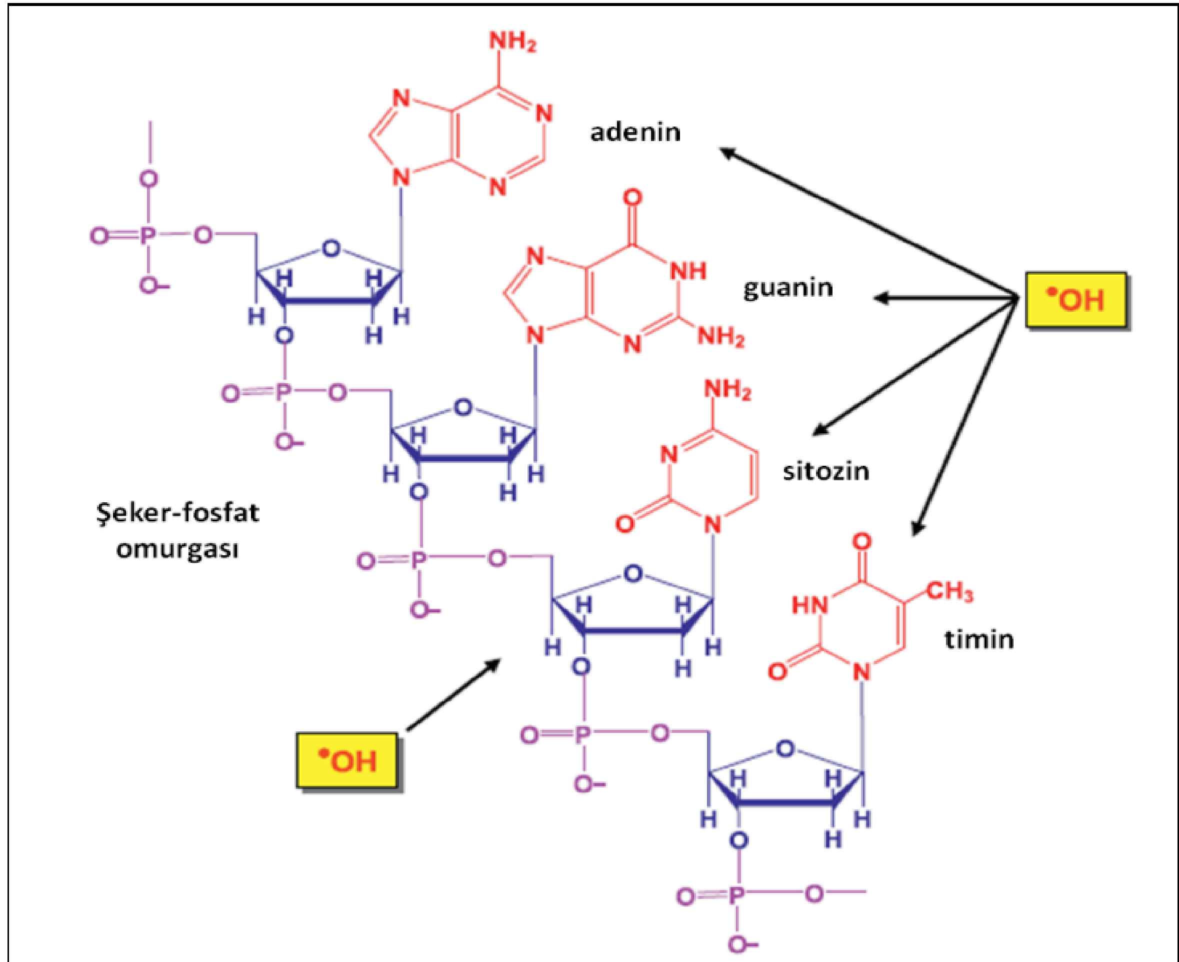
2.1. Oksidatif DNA Hasarı

Reaktif oksijen türleri, enerji üretimi sırasında ortaya çıkan yan ürünlerdir (19). Kararsız biyokimyasal yapıları nedeniyle hücrelere saldırarak, hücre hasarına neden olurlar (19). Reaktif oksijen türlerinin artması ve antioksidan sistemlerin yetersiz kalması sonucu oksidatif dengenin oksidatif stres lehine bozulduğu durumda serbest radikaller, yağ asitleri, proteinler ve DNA ile reaksiyona girerek hasara neden olmaktadır (20) (Şekil 1).



Şekil 1. DNA hasarı ve hastalıklar

Guanin, DNA bileşenleri içerisinde en düşük iyonizasyon potansiyeline sahip olan ve oksidasyona en yatkın olan bazdır. Hasara uğrayan bazlar arasında 8-hidroksiguanin (8-OHGua) en sık karşılaşılan oksidatif DNA hasarı belirtecidir ve oksidatif DNA hasarının duyarlı bir göstergesidir (20). 8-OHdG ölçümü, DNA'daki oksidatif hasarın doğrudan göstergesi olarak kabul edilmekte ve oksidatif DNA hasarını belirlemede en sık kullanılan yöntem olarak uygulanmaktadır (6). Hidroksil radikalinin DNA bazlarına eklenmesi sonucu guanin bazından C4-OH, C5-OH ve C8-OH-eklenti radikalleri, adeninden ise C4-OH ve C8-OH-eklenti radikalleri oluşur (Şekil 2).

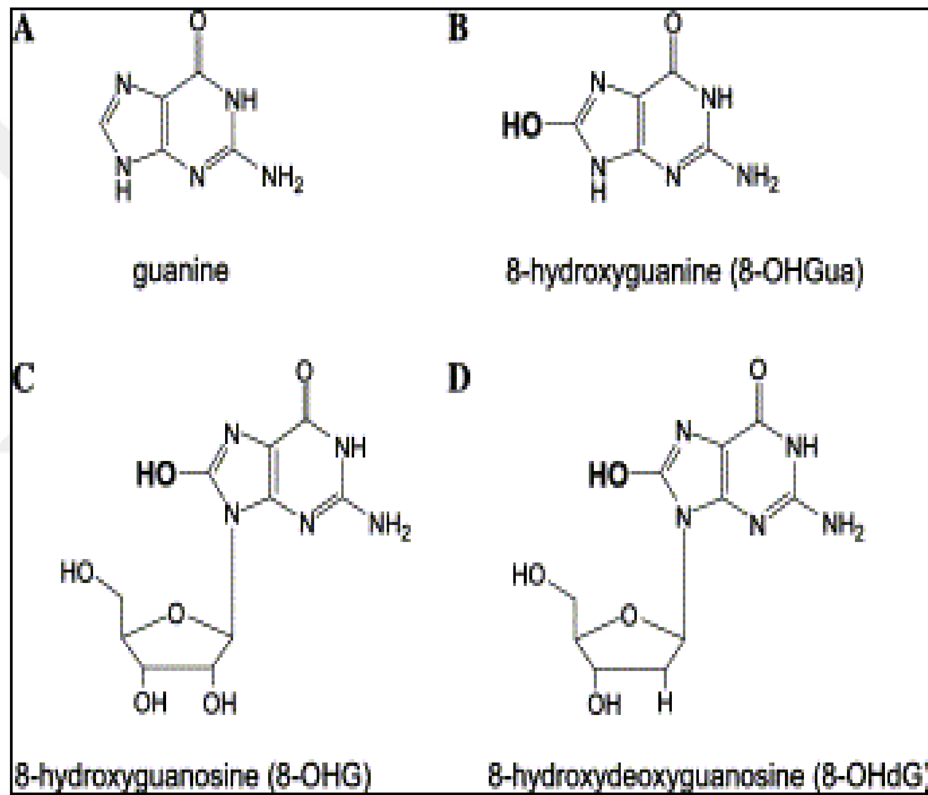


Şekil 2. Hidroksil radikalinin (.OH) DNA üzerindeki atak bölgeleri (Dizdaroğlu, 2012)

C8-OH eklenti pürin radikallerinden 8-hidroksiadenin (8-OH-Ade) ve 8-hidroksiguanin (8-OHGua) oluşur (6). Şeker radikalinin aynı pürin nükleozitindeki C8 pozisyonuna eklenmesi ile oluşan lezyonlardır. Bu molekül içi halkalanmayı oksidasyon takip eder ve 8,5'-siklo-2'-deoksiguanozin (cdG) ve 8,5'-siklo-2'-deoksiadenozin (cdA) ardışık lezyonları oluşur (Şekil

3). Oksijenin varlığında üretilmezler çünkü oksijenin C5’-merkezli radikal ile yaptığı difüzyon kontrollü reaksiyon önceliklidir (6).

Oksidatif DNA hasarı DNA zincirinin kırılmasına, nükleotid kaybına ve nükleotidlerdeki bazlarda modifikasyonlara yol açar (6). Oksidatif hasar sonucunda genetik materyalin sürekli olarak modifikasyonu, mutagenez, karsinogenez ve yaşlanmanın ilk basamağını teşkil eder (Dizdaroğlu, 2012). Kardiovasküler hastalıklarda, nörodejeneratif hastalıklarda, alkol bağımlılığında, şizofrenide ve duygudurum bozukluklarında oksidatif DNA hasarında artış gösterilmiştir (6,21,22).



Şekil 3. Guaninden modifiye nükleozit 8-OHdG’nin oluşumu (9)

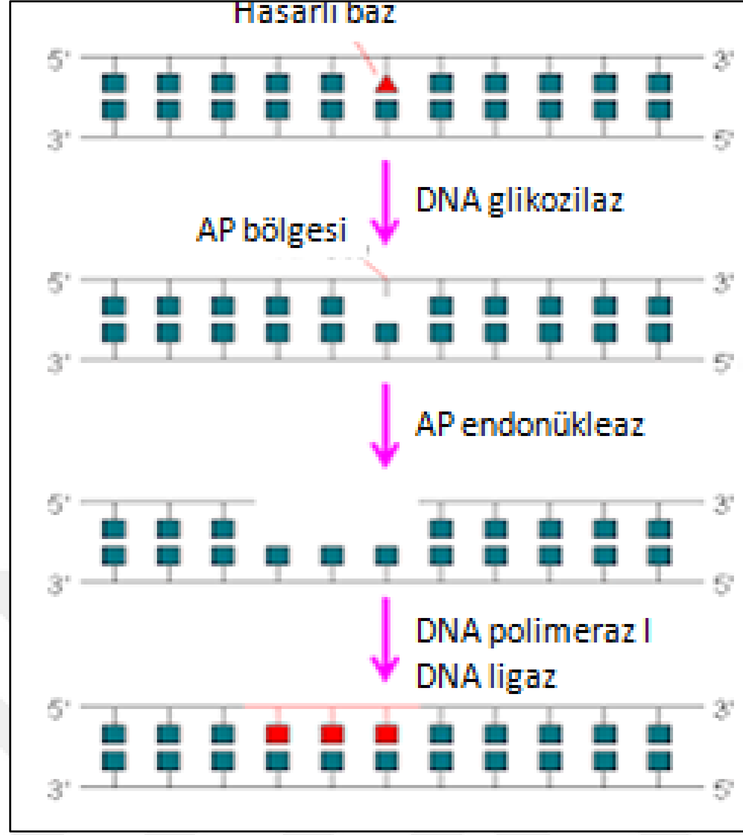
2.2. DNA Onarımı

DNA hasarı ile ilgili yapılmış araştırmalarda önemli bir eksiklik DNA onarım süreçlerinin hasar parametreleri ile birlikte çalışılmamış olmasıdır. Oksidatif DNA hasarı sağlıklı bireylerde de saptanan, çeşitli onarım sistemlerinin sürekli olarak devrede bulunduğu dinamik bir süreçtir. İnsan vücudunun her hücresindeki DNA’ların günde 103 kez oksidatif hasara maruz kaldığı öne sürülmektedir (19). DNA hasarı ve onarımı arasındaki denge

nedeniyle, sağlıklı bireylerde hasar düşük düzeylerde saptanmaktadır.

Hücreler, doğrudan onarım, baz kesip çıkarma onarımı, nükleotit kesip çıkarma onarımı, yanlış eş onarımı, DNA çift zincir kırığı onarımı ve zincirler arası çapraz bağların onarımı gibi çeşitli onarım mekanizmaları kullanmaktadır (6). Baz kesme-çıkarma onarımı (Base Exision Repair, BÇO), DNA bazlarının doğal hidrolizi veya uygun olmayan bazların onarımı ile ilgilidir (6). BÇO hasarlanmış DNA bazlarını kesip çıkaran mekanizmadır ve oksidatif hasar sonucu oluşan baz değişikliklerine özgüdür.

BÇO, DNA'daki uyumsuz bazların glikozilazlar tarafından tanınmasıyla başlamaktadır. DNA glikozilazlar hasarlı baz ile nükleotitin şeker birimi arasında bulunan N-β glikozidik bağını kırarak bazı polinükleotitten ayırmaktadır (Şekil 4). Oluşan bazsız bölge apürinik/ apirimidinik bölge olarak adlandırılmaktadır. Apürinik/ apirimidinik bölgedeki fosfodiester bağlarının endonükleazlar tarafından yıkılmasının ardından poli ADP-riboz polimeraz-1 enzimi kırık DNA uçlarına bağlanarak bu uçları yıkımdan korumaktadır. Poli ADP-riboz polimeraz-1 enzimi aktive olduktan sonra BÇO mekanizmasında fonksiyon gören enzimlerin aktivasyonunu sağlamaktadır. Apürinik/ apirimidinik bölgesinin bağlanması DNA polimeraz β tarafından gerçekleştirilir. BÇO'nın en son basamağında ise oluşan boşluk DNA polimeraz β ile doldurulur ve DNA ligaz ile fosfodiester bağı oluşturulur (23).



Şekil 4. DNA baz çıkarma onarımı (BÇO) (23)

Kromozom 3p26.2'de yer alan OGG-1 geni, α-OGG-1 ve β-OGG-1 DNA glikozilazlarını kodlamaktadır (25). OGG-1 ekspresyonunun en fazla beyinde olduğunu ve OGG-1'in guanin baz hasarını kesip çıkarmaktan sorumlu olduğu bildirilmiştir (26).

2.3. Depresyonda DNA Hasarı ve Onarımı

Güncel bir metaanaliz çalışmasında, bir nükleozid hasar belirteci olan 8-OHdG'nin depresyonda artmış olduğunu gösterilmiştir (7). Araştırmaya dahil edilen hasta grubunun klinik özelliklerine, kullanılan örnek tipine, ölçülen hasarın tipine ve ölçüm yöntemlerine bağlı olarak sonuçlarda farklılıklar ortaya çıkabileceği öne sürülmüştür (7). Araştırma grubumuza ait bir çalışmada, bipolar bozukluk hastalarında 8-OHdG düzeylerinin hastalığın manik ve depresif dönemlerinde arttığı gösterilmiştir (8).

Akut lösemi hastalarının depresif belirtisi olanlar ve olmayanlar olarak birbirleri ve sağlıklı kontrollerle karşılaştırıldığı bir araştırmada depresif hastalarda, hasarlı guanin bazlarını kesip çıkarmak için özelleşmiş OGG-1 ekspresyonunda artış, mononükleer hücrelerde PZR

ölçümleriyle gösterilmiştir (14). Depresif belirtileri olan gastrik adenokarsinom hastalarını depresif duygudurum belirtisi olmayan gastrik adenokarsinom hastaları ile karşılaştıran bir araştırmada ise depresif duygudurum belirtileri olan hastalarda hem DNA hasarında, hem de OGG-1 ekspresyonunda artış saptanmıştır (15).

Araştırma grubumuz tarafından yapılmış bir çalışmada, ötimik durumdaki bipolar bozukluk hastalarında OGG-1 ekspresyonları sağlıklı kontrollere göre azalmış olarak saptanmıştır (16). Araştırma grubumuzun bu bulgusuyla uyumlu olarak, hızlı döngülü bipolar bozukluk hastalarını inceleyen bir başka araştırmada, hızlı döngülü bipolar bozukluk hastalarının hastalık dönemlerinden bağımsız olarak OGG-1 ekspresyonu sağlıklı kontrollerden düşük saptanmıştır (17).

3. GEREÇ VE YÖNTEM

3.1. Araştırmanın Tipi

Bu araştırma iki aşamalı planlanmış tanımlayıcı bir çalışmadır. İlk bölümü olgu, kontrol deseninde bir çalışmadır. İleriye yönelik bölümünde yalnızca hasta grubu 12 hafta izlenerek, atak ve remisyon durumlarında inflamatuvar mekanizmaların değişimi incelenmiştir. İleriye dönük bölümünde çalışmaya alınan hastalar çalışmadan bağımsız olarak klinisyenin önerdiği ve durumlarının gerektirdiği tedavilerini almışlardır. Araştırmacıların hastanın tedavi sürecine bir etkisi olmamıştır.

3.2. Araştırmanın Yeri ve Zamanı

Araştırma İzmir Ekonomi Üniversitesi Sağlık Meslek Yüksekokulu, Dokuz Eylül Üniversitesi Sağlık Bilimleri Enstitüsü Sinirbilimler AD, Dokuz Eylül Üniversitesi Psikiyatri AD, Dokuz Eylül Üniversitesi Biyokimya AD işbirliği ile gerçekleştirilmiştir. Araştırma 02/02/2017-15/02/2019 tarihleri arasında gerçekleştirilmiştir.

3.3 Araştırmanın Evreni ve Örneklemi/Çalışma Grupları

Araştırmaya psikiyatri kliniklerine başvuran hastalardan, psikiyatrik tanıları Amerikan Psikiyatri Birliği DSM-IV'ün (Diagnostic and Statistical Manuel) standardize edilmiş ve yapılandırılmış görüşmesi (SCID-I) ile koyulmuş, "Bipolar bozukluk, depresif epizod", "Major depresif bozukluk, tek episod", "Major depresif bozukluk, yineleyici" tanılı her iki cinsiyetten hastalardan içleme ve dışlama kriterlerine göre araştırmaya uygun bulunanlar dahil edilmiştir.

"Open Epi" örneklem büyüklüğü hesaplama yöntemi kullanılarak, idrar 8-OHdG belirteci açısından %95 güven aralığında ve %95 güç düzeyi elde edebilmek üzere, hasta ve sağlıklı gönüllü gruplarına en az 53'er kişinin alınması gerektiği hesaplanmıştır. İzlem sırasında ve laboratuvar sürecinde ortaya çıkabilecek kayıplar göz önünde tutularak hasta ve sağlıklı gönüllü gruplarına 60'ar kişi alınması planlanmıştır. Araştırmanın etik kurul izni gerekli durumlarda kayıpların yerine yeni katılımcıların alınabilmesine uygun olarak düzenlenmiştir.

3.3.1. Çalışmaya alınma ölçütleri:

Çalışma için yazılı onam verebilen, en az ilkokul mezunu olan, Hamilton Depresyon Ölçeği puanı 18 ve/veya üzerinde olan, Hasta grubu için SCID-I ile "Bipolar bozukluk, depresif epizod", "Major depresif bozukluk, tek episod", "Major depresif bozukluk, yineleyici" tanılarından birini almış 18-45 yaş aralığında depresyon hastaları, ve SCID-I ile herhangi bir

psikiyatrik tanı almamış, hiç psikiyatrik tedavi görmemiş, muayene sonunda dışlanma ölçütlerinden herhangi bir ölçüte sahip olmadığı tespit edilmiş, hasta grubuyla aynı sayıda ve yaş diliminde yer alan sağlıklı bireyler araştırmaya dahil edilmiştir.

3.3.2. Çalışmadan dışlanma ölçütleri

Bilinen dekompanse sistemik bir tıbbi hastalık, diabetes mellitus, romatolojik bir hastalık, aktif enfeksiyon ya da ciddi bir nörolojik hastalığın bulunması, oksidatif parametreleri etkileyebilecek antioksidan içerikli tedaviler, destekleyici ürünler kullanılması, rutin laboratuar bulgularında ciddi bozukluklar olması, yaşam boyu mental retardasyon, herhangi bir eksen bir psikiyatrik hastalık, bilişsel işlevleri etkileyen durumlar (deliryum, demans, epilepsi vb.) olması, yaşamının herhangi bir döneminde alkol ve madde kullanım bozukluğu tanısının bulunması araştırmadan dışlanma nedenleridir.

3.3.3. Çalışmadan çıkarılma ölçütleri

Gönüllünün kendisinin araştırmadan ayrılmak istemesi, çalışma izlem şemasının gereklerini yerine getirmemesi, çalışma programını aksatması, izlem sırasında tedavi gerektirecek sistemik bir tıbbi hastalığın (diabetes mellitus, romatolojik bir hastalık, aktif enfeksiyon, nörolojik hastalık vb.) ya da mani atağının ortaya çıkması araştırmadan çıkarılma sebepleri olarak tanımlanmıştır.

3.3.4. Araştırmanın çalışma grupları

Çalışmamıza 145 (72 depresyon, 73 sağlıklı gönüllü) katılımcı dahil edilmiştir. Sağlıklı gönüllülerden 1 kişi çalışmadan çıkma hakkını kullandığı için, 1 kişi yüksek alkol kullanımı, 10 kişi incelemeler sırasında kendisinde ya da ailesinde ek bir hastalık tespit edildiği için dışlanmıştır. Depresyon hastalarından 6 kişi incelemelerinde ek hastalık tespit edilmesi,1 kişi depresif epizodun manik epizoda kayması, 1 kişi yüksek dozda alkol kullanımı, 1 kişi okuma yazma düzeyinin aydınlatılmış onam vermek için yetersiz oluşu, 6 kişi depresyon şiddetinin yeterli olmaması (HAD < 18) nedenleriyle dışlanmıştır.

3.4. Çalışma Materyali

Çalışmaya davet edilen hastalara idrar kabı verilerek ölçümlerin yapılacağı gün sabah ilk idrarlarını yanlarında getirmeleri istenmiştir. Ölçüm günü görüşmeleri ve ölçek değerlendirmeleri tamamlandıktan sonra katılımcılardan bir tüp kan (10 ml) alınmıştır. Örnek alımının standart koşullarda olmasının sağlanması için örnekler aç karna, sabah saat 08.00-

09.00 arasında yapılmıştır. Alınan idrar örneğinin günün ilk idrarı olduğu teyit edilmiştir. Kan işlemi araştırmacımızın eşliğinde Dokuz Eylül Üniversitesi Merkez Laboratuvarı'nda gerçekleştirilmiştir.

3.5. Araştırmanın değişkenleri:

İlk aşama için (olgu kontrol)

Sonuç (bağımlı) değişken:

1. İdrar 8-OHdG/ kreatinin düzeyleri
2. GZ-PZR ile incelenmiş OGG-1 ekspresyon düzeyleri

Temel bağımsız değişkenler:

1. Depresif bozukluk tanısı
2. Depresyonun tanısal özellikleri (bipolar depresyon/ unipolar depresyon)

Diğer tanımlayıcı ve analitik bağımsız değişkenler

1. Demografik veriler (cinsiyet, yaş vb.)
2. Klinik veriler (yaşam biçimi özellikleri, ilaç tedavileri vb.)
3. Belirti şiddeti (Hamilton Depresyon Ölçeği, Young Mani Ölçeği)

İkinci aşama için (ileriye yönelik izlem çalışması)

Sonuç (bağımlı) değişken:

1. İdrar 8-OHdG/ kreatinin düzeyleri
2. GZ-PZR ile incelenmiş OGG-1 ekspresyon düzeyleri

Temel bağımsız değişkenler:

1. Remisyon veya atakta kalma (remisyon 17-maddeli Hamilton Depresyon Ölçeği toplam puanının ≤ 7 olması)
2. Depresyonun tanısal özellikleri (bipolar / unipolar depresyon)

3.6. Veri Toplama Araçları

3.6.1. Klinik Değerlendirme Ölçümleri:

1. DSM-IV için Yapılandırılmış Klinik Görüşme: First ve arkadaşları tarafından DSM-IV Eksen I bozuklukları için yapılandırılmış klinik görüşmedir. SCID-I tanısal değerlendirmenin standart biçimde uygulanmasını sağlayarak tanının güvenirliliğinin ve geçerliliğinin arttırılması, belirtilerin sistematik olarak araştırılması için geliştirilmiştir (26).

2. Hamilton Depresyon Derecelendirme Ölçeği, 17 maddelik versiyonu: Hamilton tarafından 1960 yılında geliştirilmiştir (27) Depresif belirtileri araştıran 17 maddeden oluşan, klinisyen tarafından uygulanan bir ölçektir. Akdemir ve ark. (1996) tarafından Türkçe güvenilirlik ve geçerlilik çalışması yapılmıştır (28).

3. Young Mani Derecelendirme Ölçeği: Manik durumun şiddetini ve değişimini ölçmeye yönelik olarak Young ve ve ark. (1978) tarafından geliştirilmiştir (29). Ölçek 11 maddeden oluşmakta ve her madde beş şiddet derecesi içermektedir. Belirtiler son 1 hafta dikkate alınarak değerlendirilir. Türkçe'de geçerlilik ve güvenilirlik çalışması Karadağ ve ark. (2001) tarafından yayınlanmıştır (30).

4. Algılanan Stres Ölçeği: Toplam 14 maddeden oluşan ölçek kişinin hayatındaki birtakım durumların ne derece stresli algılandığını ölçmek için tasarlanmıştır. Yüksek puan kişinin stres algısının fazlalığına işaret etmektedir. Ölçeğin Türkçe geçerlik ve güvenirlik çalışması Eskin ve ark. (2013) tarafından yapılmıştır (31).

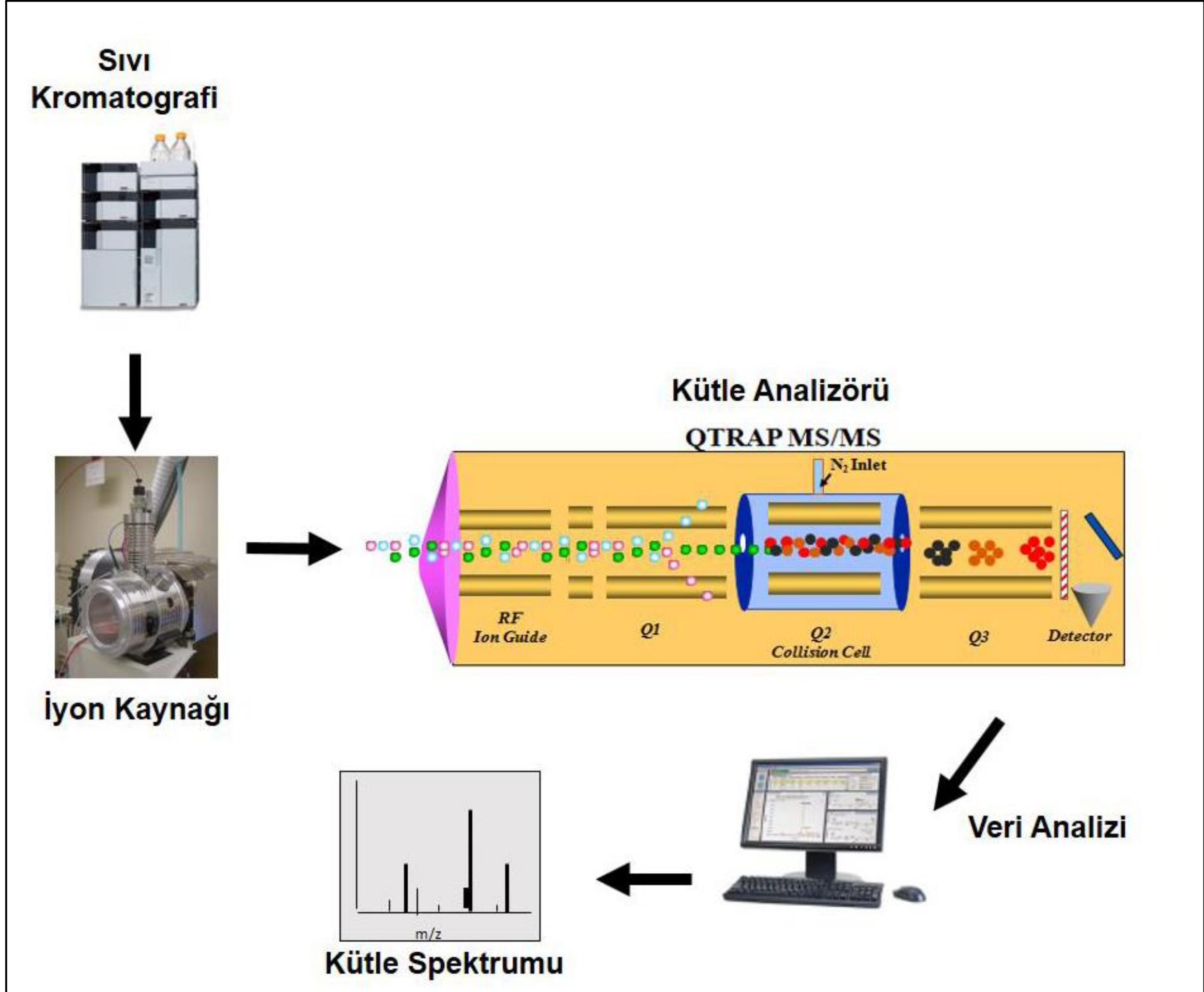
5. Hipomani Soru Listesi-32-Yenilenmiş Sürümü: Hipomani Soru Listesi-32, 32 maddeli bir özbildirim ölçeğidir. Öncelikle bir maddede genel duygudurumu soran 7-Likert tipi genel değerlendirme sonrası, bireyin duygudurum belirtilerini "taşkınlık/enerji artışı" ve "riskli davranışlar/dürtüsellik" adlı iki boyutta toplam 32 evet-hayır biçiminde soruyla araştıran form söz konusudur. Ölçeğin Türkçe geçerlik, güvenirlik çalışması Vahip ve ark. (2016) tarafından yapılmıştır (32).

6. Sağlıklı Yaşam Biçimi Davranışları Ölçeği-II: Walker vd (1987) tarafından geliştirilen ölçek 1996 yılında yeniden revize edilerek Sağlıklı Yaşam Biçimi Davranışları Ölçeği-II olarak adlandırılmıştır (33). Ölçek 52 maddedir ve altı faktörden (sağlık sorumluluğu, fiziksel aktivite, beslenme, manevi gelişim, kişilerarası ilişkiler ve stres yönetimi) oluşmaktadır. Ölçeğin tamamından alınabilecek en düşük puan 52, en yüksek puan 208'dir. Türkçe geçerlik ve güvenirlik çalışması, Bahar ve ark. (2008) tarafından yapılmıştır (34).

3.6.2. LC-MS/MS analizleri:

Bu çalışmada hasarlı DNA nükleozidi 8-OHdG ölçümlerini sıvı kromatografi-tandem kütle spektrometre (LC-MS/MS) ile Dokuz Eylül Üniversitesi Tıbbi Biyokimya Anabilim Dalı Araştırma Laboratuvarı'nda gerçekleştirilmiştir. İdrar ile atılan hasarlı DNA nükleozidlerinin ölçümünde sıklıkla kullanılan yöntemler, immunokimyasal teknikler, HPLC-ECD, GC-MS LC-MS ve LC-MS/MS'dir. Son yıllarda LC-MS/MS yöntemlerindeki gelişmeler nedeniyle 8-OH-dG'nin kantitatif ölçümünde bu yöntemin kullanımı giderek artmaktadır; yakın gelecekte

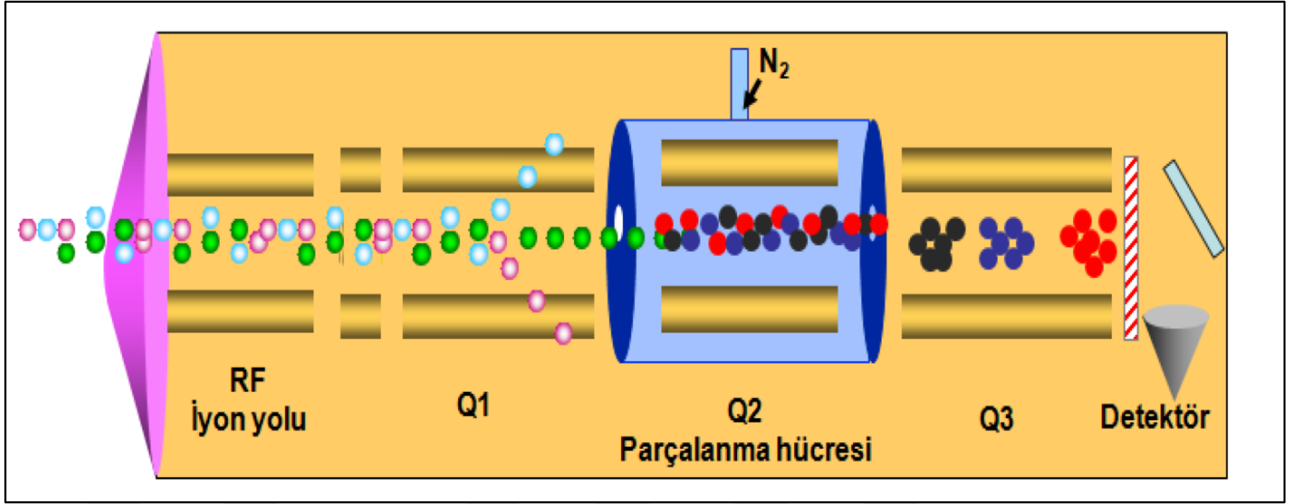
de yüksek duyarlılık, özgüllük ve güvenilirlikle aynı anda birçok analitin kantitasyonunu gerçekleştirebilme kapasitesi nedeniyle tercih edilen bir yöntem olacaktır. Avrupa Oksidatif DNA Hasarı Standartlar Komitesi (ESCODD) 8-OHdG düzeylerinin farklı tekniklerle ölçümlerinin karşılaştırması sonucunda, en güvenilir yöntemin LC-MS/MS olduğu yönünde görüş bildirmiştir (6). LC-MS/MS'in temel bileşenleri Şekil 5'de gösterilmiştir (Şekil 5).



Şekil 5. Tandem kütle spektrometrisinin bileşenleri

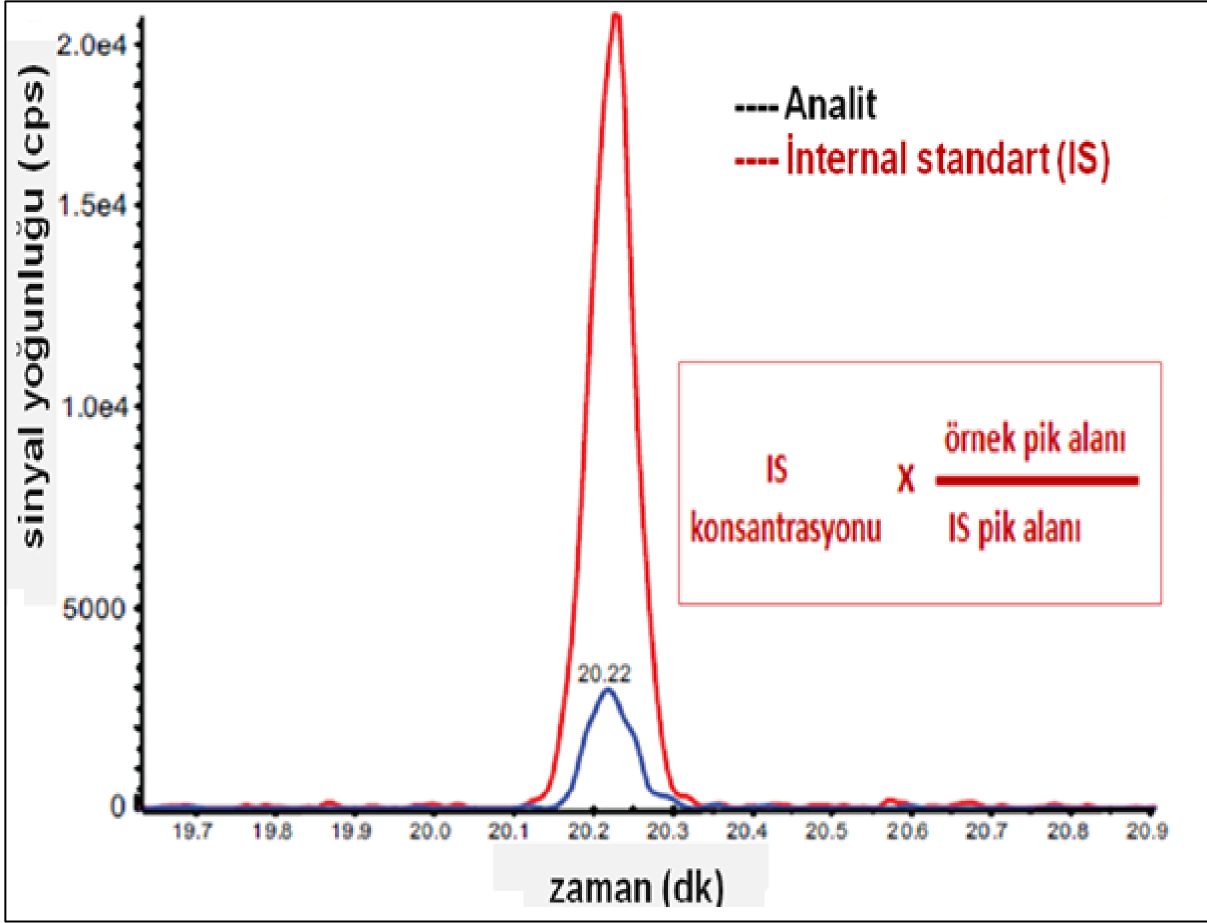
Kompleks biyolojik karışımlar HPLC kullanılarak kromatografik olarak bileşenlerine ayrılır ve ardından nitrojen gazı ve yüksek sıcaklık etkisiyle sıvı fazda bulunan bileşikler iyonlar haline gelir. Kütle analizöründeki elektriksel alan belirli iyonların geçişine izin verirken, istenmeyen diğer iyonları tutarak bir filtre görevini yapar. İki kütle analizörünün art arda geldiği sisteme sıralı kütle analizörü (Tandem MS) adı verilmektedir. Çalışmamızda kullanılan triple quadrupole-lineer iyon trap özelliğinde tandem kütle analizörü, analitlerin mutlak

kantitasyonuna ve nitel karakterizasyonuna olanak sağlayan bir hibrid kütle analizörüdür (Şekil 6).



Şekil 6. Triple quadrupole iyon trap MS/MS

Triple quadrupole kütle analizöründe çoklu tarama modu kullanılmıştır. Çoklu tarama modunda, birinci quadrupole (Q1) sırasısında önceden tanımlanmış m/z değerindeki moleküllere ait ana iyonlar seçilir, ikinci quadrupole (Q2) sırasında seçilmiş iyonlar çarpışarak parçalanır, üçüncü quadrupole (Q3) sırasında parçalanan ürün iyonlardan tanımlanmış olan ürün iyonu seçilir. Seçilen ana iyon/ürün ikilisi dedektöre ulaştığında m/z değerine karşı iyonların sinyal yoğunluğu kütle spektrumu olarak kaydedilir. Seçilen ana iyon/ürün ikilisi (transition) dedektöre ulaştığında m/z değerine karşı iyonların sinyal yoğunluğu (intensite) kütle spektrumu olarak kaydedilir. Kantitasyonun güvenirliliğini arttırmak amacıyla çalışmanın ilk aşamasında her bir örneğe belirli konsantrasyonda stabil izotoplarla işaretlenmiş internal standart eklenerek MS öncesi (ekstraksiyon, çöktürme, filtreleme gibi ön hazırlık işlemleri), MS koşullarındaki (iyonizasyon gibi) değişikliklerin standardizasyonu sağlanır ve aynı zamanda eklenen internal standardın konsantrasyonundan yararlanılarak analitin mutlak kantitasyonu gerçekleştirilir (Şekil 7).



Şekil 7. İnternal standart ve analitin kütle spektrumu

DNA nükleozidlerinin ekstraksiyonu için katılımcıların sabah ilk idrar örneklerinden elde edilen 1 mL idrar örneği içine stabil izotoplar ile işaretlenmiş internal standart 8-OH-dG15N5 eklenmiştir. İdrar örnekleri 1000 g'de 15 dk santrifüjlenmiş ve süpernatanlar filtrelenerek katı faz ekstraksiyon kartuşları ile ekstrakte edilmiştir. Ekstrakte edilen örnekler SpeedVac ile kurutulduktan sonra alkalen fosfataz ile 37°C'de 1 saat inkübe edilmiştir. İnkübasyon sonrasında 14.000 g'de 10 dk santrifüjlenen örnekler LC-MS/MS analizi öncesinde ultrafiltrasyon membranları ile filtrelenmiştir.

Hasarlı DNA nükleozidlerinin analizleri triple quadrupole ion trap tandem kütle spektrometresinde çoklu reaksiyon izleme (MRM) modunda turbo V iyon sprey kaynağı ile gerçekleştirilmiştir. Veri analizi için Analyst Software Version 1.5 kullanılmıştır. Örnekler reversed faz C18 kolonu (Zorbax SB Aq 2.1x150mm, 3.5µm) ve guard kolonu (Agilent Eclipse XDB C8 2.1x12.5mm, 5 µm) ile 0.3 mL/dk akış hızında ayrımlanmıştır. Mobil faz olarak % 0.1 formik asit içeren dH2O (A) ve asetonitril (B) kullanılarak gradient analiz

gerçekleştirilmiştir.

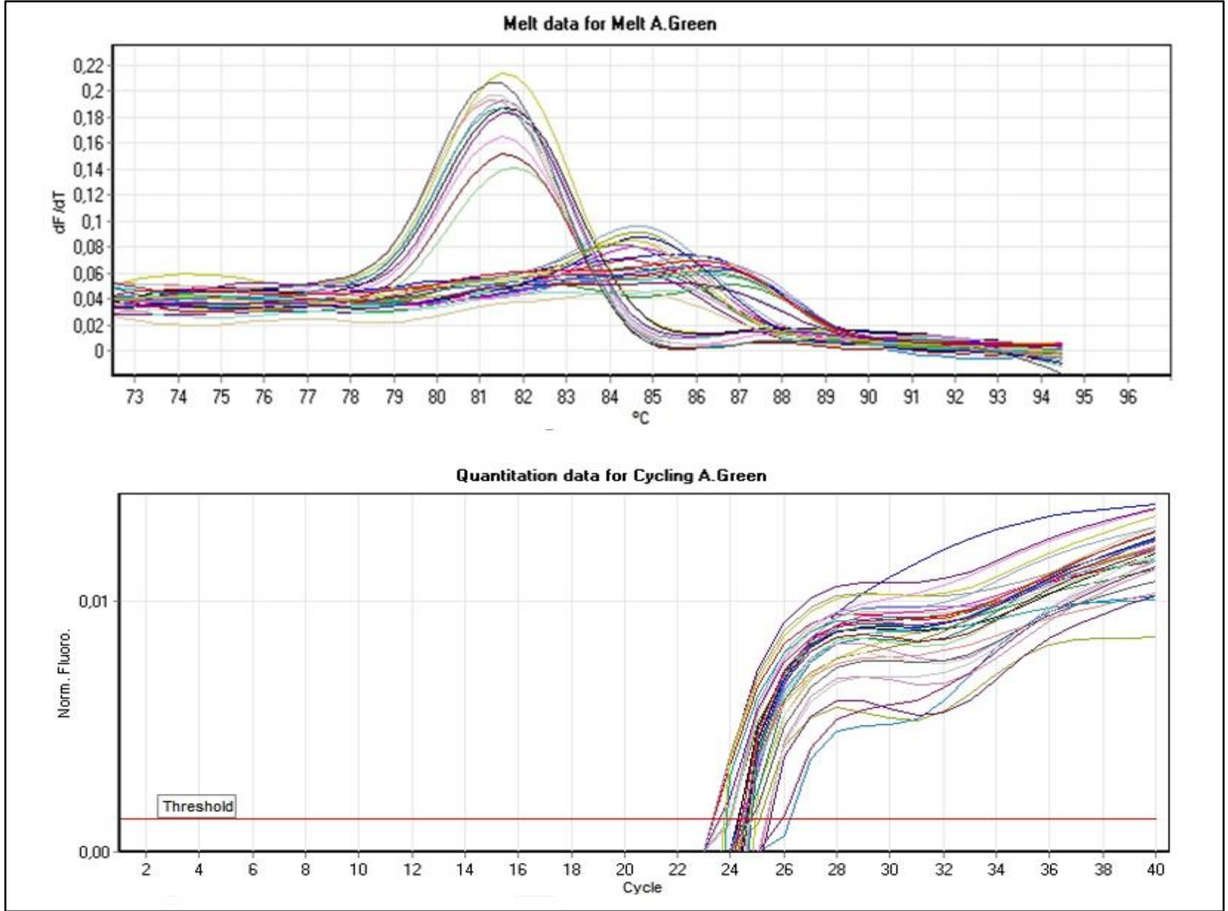
İdrar örneklerinde 8-OHdG'nin kantitasyonu için elde edilen veriler idrar kreatinin değerlerine göre normalize edilmiştir. İdrar kreatinin miktarları Dokuz Eylül Üniversitesi Merkez Laboratuvarı'nda hesaplanmıştır. Laboratuvar analizleri örneklere kör araştırmacılar tarafından yapılmıştır.

3.6.3. OGG-1 mRNA Ekspresyon Analizleri:

Toplam kandan RNA izolasyonu QIAamp RNA Blood Mini Kit (Cat No./ID: 52304, Qiagen, Almanya) ile üretici firma protokolüne uygun olarak yapılmıştır. RNA konsantrasyonları Nanodrop ile belirlenmiş ve RNA için OD260/ OD280 oranı 1,8-2,1 aralığında olan örnekler cDNA sentezinde kullanılmıştır. cDNA sentezi, cDNA Synthesis Using the RT 2 First Strand Kit (Cat No./ID: 330404, Qiagen, Almanya) ile üretici protokolüne uygun olarak gerçekleştirilmiştir. cDNA'lar -20ºC'de depolanmıştır.

Human 8-Oxoguanine Glycosylase 1 (OGG-1) için primer sekansları P1: 5'-CAAGTGCTGGGATCAAAGGTG-3'; P2: 5'-GCTCCTTCTTGTAGCCGACG-3'; P3: 5'-TCCTCGTGCTTTACGGTATCG-3' olarak belirlenmiş ve üretici firma tarafından sentezlenmiştir (Qiagen, Almanya).

Housekeeping genler olarak GAPDH ve ACTB kullanılmıştır (Qiagen, Almanya). Gen ekpresyonu Rotor-Gene Q (Qiagen, Almanya) (DEÜ Tıp Fak. ARLAB) GZ-PZR cihazı kullanılarak yapılmıştır (Şekil 8). PZR işlemi SYBR Green Master Mix (Qiagen, Almanya) ve GZ-PZR cihazı üretici firmasının protokolüne uygun olarak çalışılmıştır (RT2 Profiler PCR Array Handbook, Qiagen).



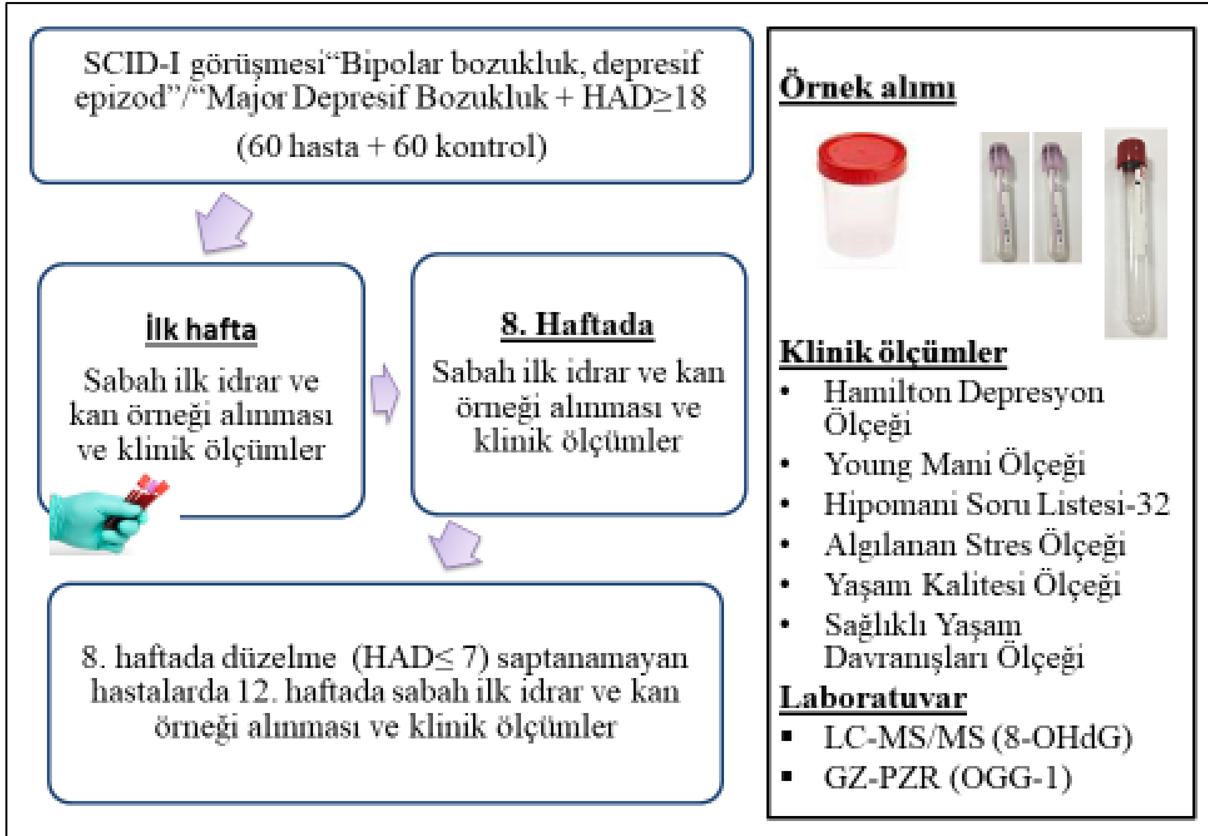
Şekil 8. OGG-1 mRNA ekspresyon düzeylerinin GZ-PZR kullanılarak incelenmesi

GZ-PZR işleminde elde edilen sonuçların değerlendirilmesinde göreceli kantifikasyon (karşılaştırmalı Ct) metodu ile; internal kontrol (Beta actin ve GAPDH) kullanılarak, PZR'nin üstel fazında amplifikasyonları karşılaştırılarak gen ekspresyon düzeyleri yorumlanmıştır (Kubista ve ark., 2006). Ct (treshold cycle) değeri eşik değere ulaşmak için gerekli döngü sayısı olarak tanımlanmaktadır. Kontrol (referans) ile araştırılan gen bölgesi arasındaki Ct (cycle of treshold) değer farklılığı 'housekeeping gen' ile normalize edilip ΔΔCt oranı hesaplanarak bulunmuştur (ΔΔCt = ΔCt örnek – ΔCt referans). Karşılaştırmalı Ct metoduna göre gen ekspresyon düzeyleri, elde edilen bu değerlerin 2–ΔΔCt formülü ile hesaplanmıştır. Laboratuvar analizleri örneklere kör araştırmacılar tarafından yapılmıştır.

3.7. Araştırma Planı ve Takvimi

Araştırma iki aşamadan oluşmuştur (Şekil 9). Birinci aşamada kesitsel olarak, depresyon hastaları sağlıklı kontrollerle karşılaştırılmıştır. İkinci aşamada, belirtilerin düzelmesi ile 8-

OHdG ve OGG-1 düzeylerindeki değişikliklerin incelenebilmesi için hastalar 8-12 hafta izlenmiştir. 8. haftada klinik ölçümlerde düzelme saptanmayan hastaların son ölçümleri 12. haftada tekrarlanmıştır. Düzelme (remisyon), hastalığın belirtilerinin ortadan kalkmış olduğuna karar verilebilecek düzeyde azalmış olmasıdır. Sıklıkla tedavi ile ortaya çıkan bir durumu ifade etmek için kullanmakla birlikte kendiliğinden de oluşabilir (spontan düzelme) (35).



Şekil 9. Araştırmanın planı

Araştırmamızda düzelme 17-maddeli Hamilton Depresyon Ölçeği toplam puanının ≤ 7 olması ve SCID görüşmesinde depresyon tanısal kriterlerinin karşılanmıyor oluşu ile tanımlanmıştır. Hastaların %60,3'ünün (s=35) izlem görüşmeleri yapılmıştır, hastaların %39,7'si (s=23) izlemden kendi istekleri ile çıkmıştır, 1 hasta izlem döneminde manik epizod geçirmesi, 4 hasta 12. haftada düzelme saptanmaması nedeniyle izlemden dışlanmıştır. İzlemler sırasında hastaların sosyodemografik verileri, depresyonla ilişkili klinik değişkenler, önceden ya da halen almakta oldukları tedaviler, beden kitle indeksi, hastanın rutin tahlillerinin sonuçları klinik değerlendirme ölçek puanları ile birlikte kaydedilmiştir.

Tablo 1. Araştırmanın takvimi

	2015	2016	2017	2018	2019
Literatür taraması ve tez önerisinin hazırlanması	X (Eylül-Aralık)				
Etik kurul izni		X (25.02.2016)			
Araştırma teşvik ödülü		X (07.05.2016)			
TÜBİTAK başvurusu		X (13.08.2016)			
TÜBİTAK bütçe onayı			X (03.09.2017)		
Araştırma örneklerinin toplanması			X Kasım 2017 – Aralık 2018		
Biyokimyasal analizler				X (Kasım- Ocak)	
Verilerin analizi ve bulguların yorumlanması					X (Ocak-Şubat)

3.8. Verilerin Değerlendirilmesi

İstatistiksel analizler için IBM SPSS 23.0 paket programı (Chicago IL, USA) kullanılmıştır. Her iki aşama için verilerin tanımlanmasında kategorik veride sayı ve yüzde, sürekli veride parametrik koşullara göre ortalama ve sapma ile ifade edilmiştir. Sürekli veriler ikili ve üçlü karşılaştırmalarda kullanılmadan önce normal dağılım açısından histogramın, eğikliğin ve basıklığın gözlenmesi ve Shapiro Wilk Testi ile kontrol edilmiştir. Normal dağılıma uygun olmayan sürekli veriler karekök dönüşümü (idrar 8-OHdG/ kreatinin değeri, eğitim süresi) ya da logaritmik dönüşüm (OGG-1 gen ekspresyonu düzeyleri, depresyonun süresi) kullanılarak normal dağılıma uygun hale getirilmiştir. Tüm istatistik karşılaştırmalarda

$p<0.05$ istatistik anlamlılık sınır değeri alınmıştır.

Olgu kontrol aşama bölümünde sosyodemografik veriler, klinik ölçümler olgu (depresyon hastaları) ve kontrollerin (sağlıklı gönüllüler) arasında, ardından depresyon alt tipleri (bipolar ve unipolar depresyon) arasında kategorik veriler için ki-kare testi, sürekli veriler için bağımsız gruplar arası t-testi kullanılarak karşılaştırılmıştır. İdrar 8-OHdG/ kreatinin düzeyleri, her bir örnek için LC-MS/MS analizinde saptanan 8-OHdG (nmol/mL) değeri, aynı örnekte saptanan kreatinin değerine (mmol/mL) bölünerek hesaplanmıştır. OGG-1 ekspresyon düzeylerini belirlemek için kullanılan ΔΔCt hesaplamasında (ΔΔCt = ΔCt örnek – ΔCt referans), ΔCt örnek değeri için her bir örneğin ΔCt değeri, ΔCt referans değeri için sağlıklıkların ortalama ΔCt değeri kullanılmıştır. İdrar 8-OHdG/ kreatinin ve OGG-1 ekspresyon düzeyleri önce depresif hastalar ve sağlıklı gönüllüler arasında bağımsız gruplar arası t-testi kullanılarak, ardından unipolar depresyon, bipolar depresyon ve sağlıklı gönüllü grupları arasında tek yönlü varyans analizi (ANOVA) kullanılarak karşılaştırılmıştır. İkili ve üçlü grup karşılaştırmalarında klinik parametrelere (yaş, cinsiyet, beden kitle indeksi, sigara içme durumu) göre düzeltmelerin yapılabilmesi için üç yönlü kovaryans analizi (ANCOVA) modelleri kullanılmıştır.

İzlem çalışması bölümünde belirtili dönem ve düzelme sonrası idrar 8-OHdG/ kreatinin düzeylerinin analizinde bağımlı gruplar t testi kullanılmıştır. OGG-1 ekspresyon düzeylerini belirlemek için kullanılan ΔΔCt hesaplamasında (ΔΔCt = ΔCt örnek – ΔCt referans) ΔCt örnek değeri için düzelme sonrası ΔCt değeri, referans değeri için belirtili dönemdeki ΔCt değeri kullanılmıştır. OGG-1 ekspresyon düzeylerinin belirtili dönem ve düzelme sonrası karşılaştırmasında örneklerin ΔCt değerleri (parametrik koşulu karşılamadığı için) Wilcoxon testi ile karşılaştırılmıştır.

DNA hasar ve onarım enzim düzeyleri arasındaki ilişki, klinik parametrelerle laboratuvar bulgularının ilişkileri korelasyon ve lineer regresyon analizleriyle incelenmiştir. Korelasyon incelemelerinde Spearman Korelasyon analizi kullanılmıştır.

Depresif belirtilerin düzelmesi sonrası 8-OHdG/ kreatinin düzeylerinin belirtili dönem 8-OHdG/ kreatinin düzeylerine oranına etki eden faktörlerin değerlendirmesi için kurulan regresyon modeline; yaş, cinsiyet, beden kitle indeksi, sigara kullanımı, ilaç kullanımı (antipsikotik, duygudurum dengeleyici), depresyonun süresi, özkıyım girişimi sayısı, klinik değerlendirme ölçekleri toplam puanları (Sağlıklı Yaşam Biçimi, Hamilton Depresyon, Young Mani, Algılanan Stres ölçekleri), belirtili dönemdeki OGG-1 ekspresyonu ve 8-OHdG/ kreatinin düzeyleri ve unipolar/bipolar depresyon tanısı değişkenlerinin bağımsız değişkenler

olarak dahil edilmiştir

Düzelme sonrası OGG-1 ekspresyonu kat değişikliğine etki eden faktörlerin değerlendirmesi için kurulan regresyon modeline; yaş, cinsiyet, beden kitle indeksi, sigara kullanımı, ilaç kullanımı (antipsikotik, duygudurum dengeleyici), depresyonun süresi, özkıyım girişimi sayısı, klinik değerlendirme ölçekleri toplam puanları (Sağlıklı Yaşam Biçimi, Hipomani Soru Listesi, Hamilton Depresyon, Young Mani, Algılanan Stres ölçekleri), belirtili dönem 8-OHdG/ kreatinin düzeyleri, unipolar/bipolar depresyon tanısı, belirtili ve düzelme sonrası dönemdeki OGG-1 ekspresyonu değerleri değişkenlerinin bağımsız değişkenler olarak dahil edilmiştir.

3.9. Araştırmanın Sınırlılıkları

Araştırmamızda bütçe sınırı nedeniyle baz çıkarma onarımında rol alan enzimler arasında 8-OHdG hasarına özgül enzim olan, ve baz çıkarma onarımını başlatan enzim olan OGG-1 incelenmek üzere seçilmiştir. Baz çıkarma yolağındaki diğer genlerin de inceleneceği ve OGG-1 enzimini farklı örneklerde protein ve enzim aktivitesi düzeyinde inceleyen yeni araştırmalar bu konudaki bilgi birikimine katkı sunacaktır. İkinci kısıtlılık araştırmanın hastaların ilaç kullanımlarının etkisini değerlendirecek bir desene sahip olmayışıdır. Psikotrop ilaçların DNA hasar ve onarım süreçlerine etkilerde bulunduğuna dönük kanıtlar mevcuttur. Ancak araştırmamızda depresyon hastalarının bazal ölçümleri üzerinden yürüttüğümüz analizde ilaç kullanımları ile bir ilişki saptanmamıştır. Düzelme dönemini izleyebildiğimiz hasta sayısı bazı alt grup analizlerinde Tip II hata riskini ortaya çıkarmıştır.

3.10. Etik Kurul Onayı

Araştırmamız Dokuz Eylül Üniversitesi Klinik Eğitim Araştırmalar Etik Kurulu'nun 25.02.2016 tarihli ve 307-SBKAEK protokol numaralı kararı ile onaylanmıştır (EK-1). Hasta ve sağlıklı gönüllülerin tamamının imzalı aydınlatılmış onamları alınmıştır (EK-2 ve EK-3).

4. BULGULAR

4.1. Katılımcıların sosyodemografik özellikleri ve klinik ölçümler

Çalışmada toplam 118 (57 depresyon hastası, 61 sağlıklı gönüllü) katılımcının örnekleri analiz edildi. Depresyon tanılı hastaların ve sağlıklı gönüllülerin sosyodemografik verileri Tablo 2'de sunulmuştur (Tablo 2). Gruplar arasında cinsiyet, medeni (evlilik) durumu, toplam eğitim süresi, aktif çalışma durumu, sigara kullanımı, alkol kullanımı ve aktif mesleki stresörlerin varlığı açısından anlamlı istatistiksel farklılık saptanmazken, yaş ortalamaları ve aktif ailesel stresörlerin varlığı açısından anlamlı farklılıklar saptanmıştır (F=0,138, t=-3,376, p=0,001; F=1,730, t=-2,223, p=0,028; χ2=23,092 df=1, p<0,001).

Tablo 2. Katılımcıların sosyodemografik özellikleri

	Depresyon hastaları (s=57)	**Sağlıklı gönüllüler (s=61)**	**İstatistiksel anlamlılık düzeyi**
Yaş (ort ± ss)	33,09 ± 8,3	27,92 ± 8,21	**F=0,381, t=-3,398, p=0,001**
Cinsiyet (s,% kadın)	42, % 75,0	38, % 61,3	χ2=2,533, df=1, p=0,081
Medeni durum (s, % evli)	24, % 42,9	17, % 27,9	χ2=2,881, df=1, p=0,090
Çalışma durumu (s, % çalışıyor)	31, % 55,4	30, % 49,2	χ2=0,446, df=1, p=0,504
Beden kitle indeksi (kg/m^2; ort ± ss)	25,04 ± 4,57	23,48 ± 3,93	F=0,907, t=-1,979, p=0,050
Düzenli egzersiz (s, % yapıyor)	15, % 27,3	27, % 44,3	χ2=3,614, df=1, p=0,057
Sigara kullanımı (s, % kullanmıyor)	27, % 48,2	33, % 54,1	χ2=0,405, df=1, p=0,525
Alkol kullanımı (s, % kullanmıyor)	33, % 58,9	26, % 43,3	χ2=2,819, df=1, p=0,093
Mesleki stresörler	41, %73,2	51, % 83,6	χ2=1,877 df=1, p=0,171

(s, % stresör yok)			
Ailesel stresörler (s, % stresör yok)	33, % 58,9	58, % 95,1	$\chi2$=22,079 df=1, p<0,001

Depresyon hastalarının sağlıklı gönüllüler ile karşılaştırıldığında istatistiksel anlamlılık düzeyinde yüksek Hamilton Depresyon Ölçeği, Young Mani Ölçeği, Algılanan Stres Ölçeği toplam puanlarına, ve istatistiksel anlamlılık düzeyinde düşük Yaşam Biçimi Ölçeği puanlarına sahip oldukları saptanmıştır (Tablo 3).

Tablo 3. Klinik özellikler ve ölçümler

	Depresyon hastaları (s=57)	**Sağlıklı gönüllüler (s=61)**	**İstatistiksel anlamlılık düzeyi**
Hamilton Depresyon Ölçeği (ort ± ss)	21,70 ± 5,29	0,44 ± 1,18	**F=49,830, t=-28,831, p<0,001**
Young Mani Ölçeği (ort ± ss)	1,38 ± 1,54	0,22 ± 0,77	**F=41,125, t=-4,928, p<0,001**
Algılanan Stres Ölçeği (ort ± ss)	31,86 ± 6,45	21,76 ± 7,01	**F=1,513, t=-7,234, p<0,001**
Hipomani Soru Listesi-32 (ort ± ss)	17,87 ± 8,66	18,20 ± 5,31	F=16,065, t= 0,226, p=0,821
Yaşam Biçimi Ölçeği (ort ± ss)	99,90 ± 22,06	134,27 ± 21,05	**F=0,001, t=7,349, p<0,001**

4.2. Depresyon hastalarının klinik özellikleri

Çalışmaya dahil edilen depresyon hastalarının (s=57) 33'ü (% 57,9) unipolar, 24'ü (% 42,1) bipolar depresyon tanısı almıştır. Hastaların 14'ü (% 24,6) ilk kez depresif epizod geçirmektedir. Hastaların aktif depresif epizodunun ortalama süresi 11,29 ± 13,01 hafta olarak saptanmıştır. Bipolar ve unipolar depresyon hastalarının sosyodemografik ve klinik özellikler açısından karşılaştırılması Tablo 4'te gösterilmiştir.

Tablo 4. Unipolar ve bipolar depresyon hastalarının klinik özellikleri

	Bipolar depresyon (s=24)	Unipolar depresyon (s=33)	İstatistiksel anlamlılık düzeyi
Yaş (ort ± ss)	32,88 ± 7,80	33,25 ± 8,77	F=2,117, t=-0,166, p=0,869
Cinsiyet (s,% kadın)	17, % 70,8	25, % 78,1	χ2=0,389, df=1, p=0,533
Medeni durum (s, % evli)	8, % 33,3	16, % 50,0	χ2=1,556, df=1, p=0,212
Çalışma durumu (s, % çalışıyor)	15, % 62,5	16, % 50,0	χ2=0,867, df=1, p=0,352
Beden kitle indeksi (kg/m^2; ort ± ss)	25,22 ± 4,42	24,91 ± 4,74	F=0,022, t=-0,252, p=0,802
Düzenli egzersiz (s, % yapıyor)	8, % 34,8	7, % 21,9	χ2=1,124, df=1, p=0,289
Sigara kullanımı (s, % kullanmıyor)	14, % 58,3	13, % 40,6	χ2=1,722, df=1, p=0,189
Alkol kullanımı (s, % kullanmıyor)	13, % 54,2	20, % 62,5	χ2=0,394, df=1, p=0,530
Mesleki stresörler (s, % stresör yok)	18, % 75,0	24, % 72,7	χ2=0,037, df=1, p=0,847
Ailesel stresörler (s, % stresör yok)	13, % 54,2	20, % 62,5	χ2=23,092 df=1, p=0,627
Depresif epizodun süresi (hafta) (ort ± ss)	7,25 ± 4,56	14,39 ± 16,15	F=5,658, t=-1,281, p=0,180
Özkıyım girişimi sayısı (ort ± ss)	0,79 ± 1,69	0,44 ± 0,80	F=1,939, t=1,040, p=0,340
Hamilton Depresyon Ölçeği (ort ± ss)	21,92 ± 6,74	21,64 ± 3,98	F=4,816, t=0,182, p=0,857
Young Mani Ölçeği	1,38 ± 1,81	1,38 ± 1,34	F=1,721, t=-0,187, p=0,852

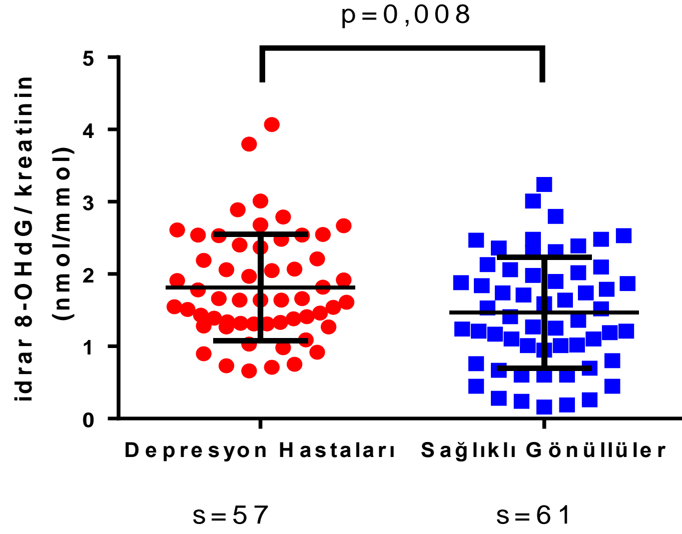
(ort ± ss)			
Hipomani Soru Listesi-32 (ort ± ss)	21,92 ± 6,73	13,72 ± 7,89	F=1,400, t=4,228, p<0,001
Algılanan Stres Ölçeği (ort ± ss)	31,65 ± 5,92	32,05 ± 6,99	F=0,000, t=-0,139, p=0,890
Yaşam Biçimi Ölçeği (toplam puan) (ort ± ss)	104,53 ± 20,57	96,48 ± 22,90	F=0,187, t=1,147 p=0,259

Depresif bozukluğu olan hastaların %73,7'si (s=42) ilaç tedavisi almaktayken, %26,3'ü (s=15) ilaç kullanmamaktaydı. Unipolar depresyonu olan hastaların 11'i, bipolar depresyonu olan hastaların 4'ü ilaç tedavisi almamaktaydı. Hastaların %42,1'i (s=24) antidepresan, %29,8'i (s=17) duygudurum dengeleyici, %26,3'ü (s=15) antipsikotik ilaç tedavisi almaktaydı.

4.3. İdrar 8-OHdG/ kreatinin bulguları

4.3.1 İdrar 8-OHdG/ kreatinin bulgularının depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

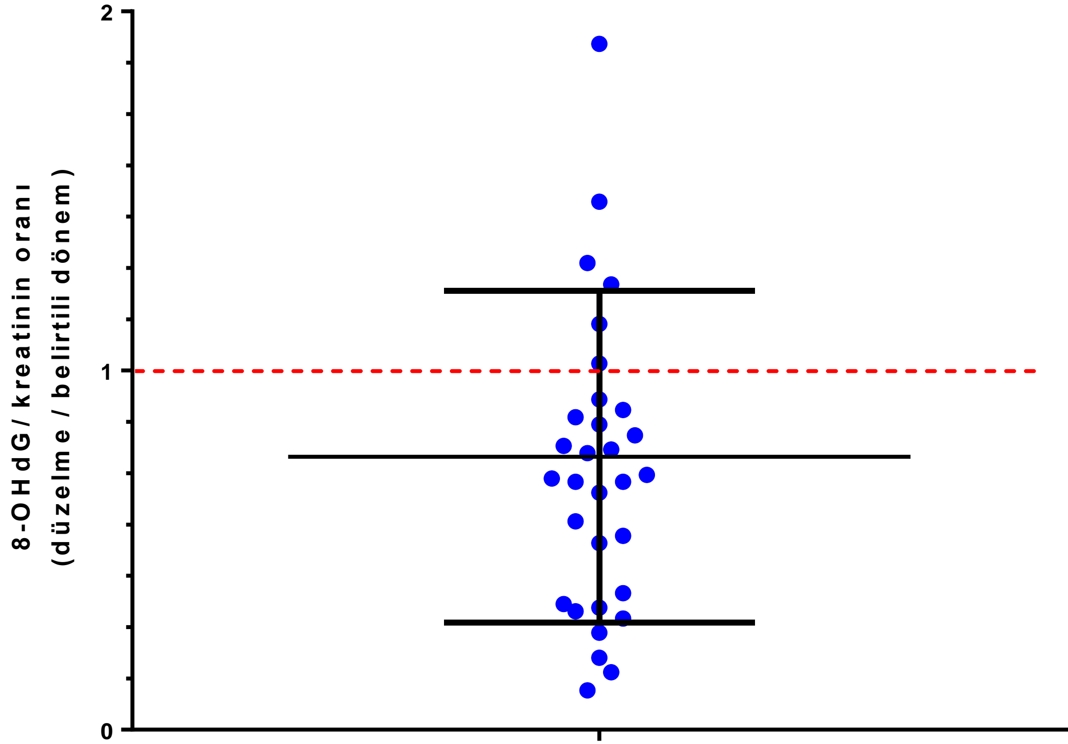
8-OHdG/ kreatinin düzeyleri ortalaması sağlıklı gönüllü grubunda 1,47 ± 0,77 nmol/mmol, depresyon grubunda 1,83 ± 0,74 nmol/mmol olarak saptanmıştır (Şekil 10). Depresyon grubunun ortalama 8-OHdG/ kreatinin düzeyleri, sağlıklı gönüllülerinkilerle karşılaştırıldığında istatistiksel anlamlılık düzeyinde yüksek olarak saptanmıştır (F=3,652, t=-2,747, p=0,007). ANCOVA analizinde yaş, cinsiyet, beden kitle indeksi, sigara içme durumu ile düzeltilerek kontrol edildiğinde, 8-OHdG/ kreatinin düzeyleri açısından depresyon hastaları ve sağlıklı kontrollerin arasındaki farklılığın anlamlı olduğu gösterilmiştir (F=7,278, df=1, p=0,008).



Şekil 10. İdrar 8-OHdG/ kreatinin bulgularının depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

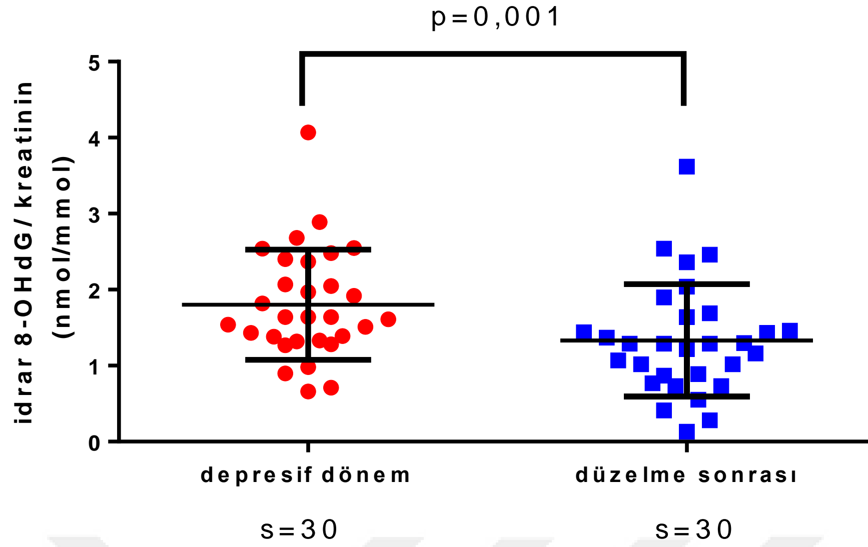
4.3.2 Depresyon hastalarının idrar 8-OHdG/ kreatinin bulgularının belirtili dönem ve düzelme sonrası arasında karşılaştırılması

Belirtili dönem ve düzelme dönemi 8-OHdG/ kreatinin ölçümleri sonuçlandırılmış olan depresyon hastalarının (s=30) 8-OHdG/ kreatinin düzeyleri analiz edilmiştir. Düzelme döneminde, belirtili döneme göre 8-OHdG/ kreatinin düzeyleri ortalama 0,47 kat azalmıştır (Şekil 11).



Şekil 11. Depresyon hastalarının idrar 8-OHdG/ kreatinin bulgularının belirtili dönem ve düzelme sonrası arasında değişimi

Depresyon hastalarının 8-OHdG/ kreatinin düzeyleri ortalaması belirtili dönemde 1,83 ± 0,74 nmol/mmol, düzelme döneminde 1,28 ± 0,75 nmol/mmol olarak saptanmıştır (Şekil 10). Depresyon hastalarının ortalama 8-OHdG/ kreatinin düzeylerinin, düzelme döneminde anlamlı olarak azaldığı saptanmıştır (t=3,585, df=29, p=0,001) (Şekil 12). Düzelme dönemi 8-OHdG/ kreatinin düzeyleri bipolar ve unipolar depresyon hastaları arasında farklılık göstermemektedir (t=-0,306, df=28, p=0,762).

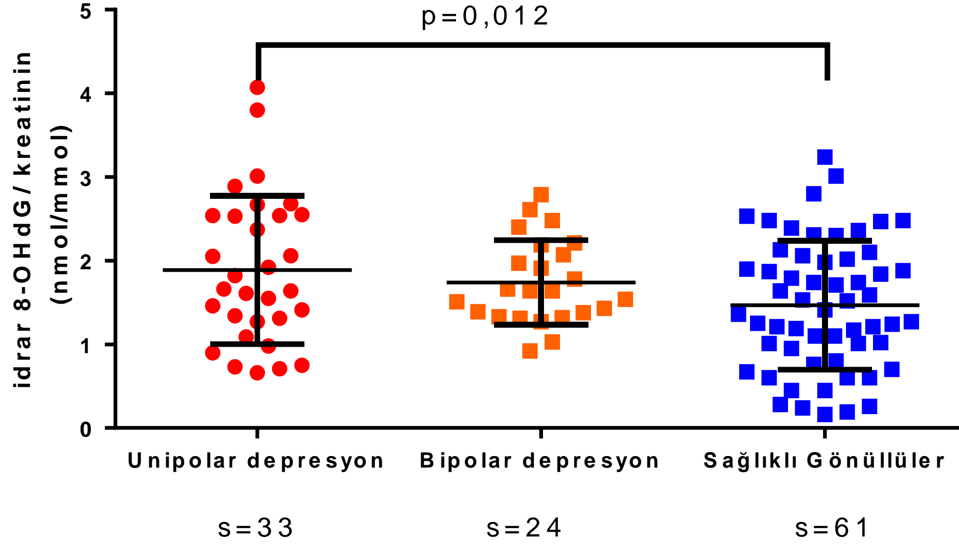


Şekil 12. Depresyon hastalarının idrar 8-OHdG/ kreatinin ortalamalarının belirtili dönem ve düzelme sonrası arasında karşılaştırılması

4.3.3 İdrar 8-OHdG/ kreatinin bulgularının bipolar depresyon, unipolar depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

8-OHdG/ kreatinin düzeyleri ortalaması sağlıklı gönüllü grubunda 1,47 ± 0,77 nmol/mmol, unipolar depresyon grubunda 1,89 ± 0,89 nmol/mmol, bipolar depresyon grubunda 1,74 ± 0,50 olarak saptanmıştır (Şekil 13). Gruplar 8-OHdG/ kreatinin düzeyleri açısından anlamlı farklılık göstermiştir (F=0,749 df=2, p=0,025). Bonferonni post-hoc karşılaştırmasında, unipolar depresyon hastalarının 8-OHdG/ kreatinin düzeyleri sağlıklı kontrollerinkilere göre anlamlı olarak yüksek saptanırken (p=0,041), bipolar depresyon hastaları ile sağlıklı kontroller arasında ve bipolar depresyon hastaları ile unipolar depresyon hastaları arasında fark saptanmamıştır.

ANCOVA analizinde yaş, cinsiyet, beden kitle indeksi, sigara içme durumu ile düzeltilerek kontrol edildiğinde, 8-OHdG/ kreatinin düzeyleri açısından üç grup arasındaki farklılığın anlamlı olduğu gösterilmiştir (F=5,305, df=2, p=0,007). Bonferonni post-hoc karşılaştırmasında, unipolar depresyon hastalarının 8-OHdG/ kreatinin düzeyleri sağlıklı kontrollerinkilere göre anlamlı olarak yüksek saptanırken (p=0,012), bipolar depresyon hastaları ile sağlıklı kontroller arasında ve bipolar depresyon hastaları ile unipolar depresyon hastaları arasında fark saptanmamıştır.

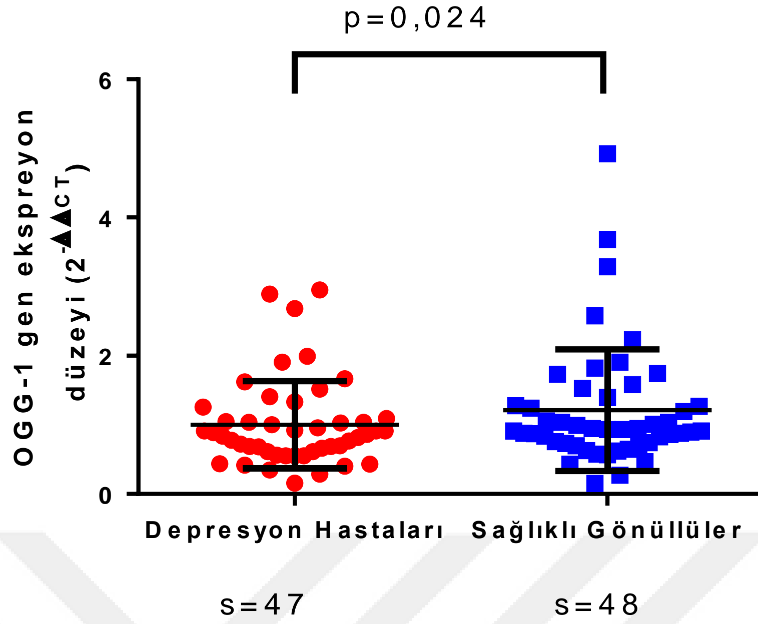


Şekil 13. İdrar 8-OHdG/ kreatinin bulgularının bipolar depresyon, unipolar depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

4.4. OGG-1 gen ekspresyonu bulguları

4.4.1 OGG-1 gen ekspresyonu bulgularının depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

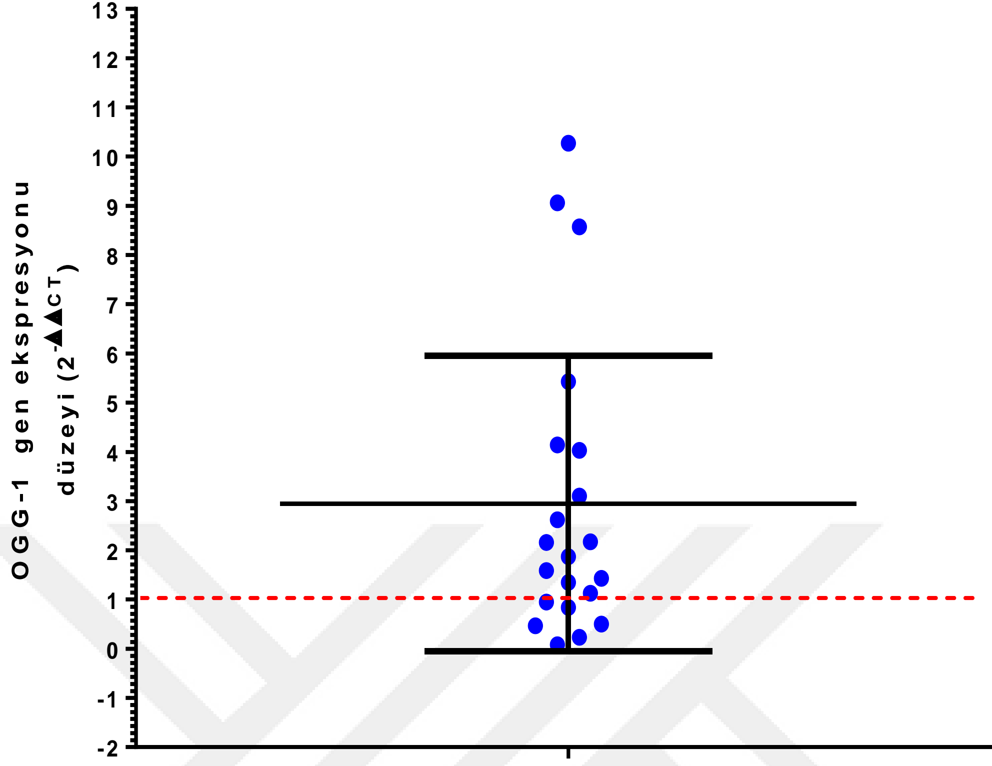
OGG-1 mRNA ekspresyon düzeyleri ortalaması sağlıklı gönüllü grubunda 1,21 ± 0,88, depresyon grubunda 0,98 ± 0,63 olarak saptanmıştır (Şekil 14). Depresyon grubunun ortalama OGG-1 geni ekspresyon düzeyleri sağlıklı gönüllülerinkilerle karşılaştırıldığında istatistiksel anlamlılık düzeyinde düşük olarak saptanmamıştır (F=0,002, t=1,549, p=0,125). ANCOVA analizinde yaş, cinsiyet, beden kitle indeksi, sigara içme durumu ile düzeltilerek kontrol edildiğinde, 8-OHdG/ kreatinin düzeyleri açısından depresyon hastaları ve sağlıklı kontrollerin arasındaki farklılığın anlamlı olduğu gösterilmiştir (F=5,299, df=1, p=0,024).



Şekil 14. OGG-1 geni ekspresyon düzeylerinin depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

4.4.2 Depresyon hastalarının OGG-1 gen ekspresyonu düzeylerinin belirtili dönem ve düzelme sonrası arasında karşılaştırılması

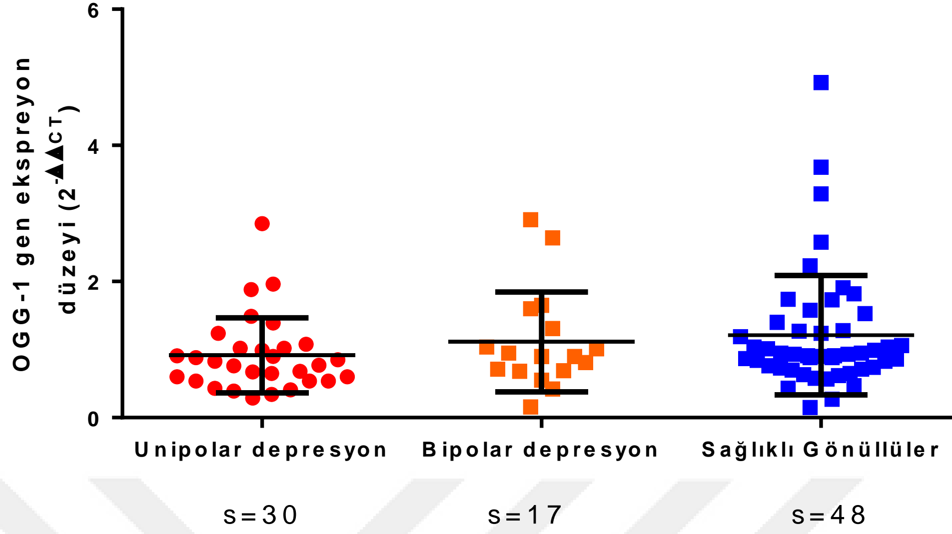
Belirtili dönem ve düzelme sonrası OGG-1 gen ekspresyonu ölçümleri sonuçlandırılmış olan depresyon hastalarının (s=21; 12 unipolar depresyon, 9 bipolar depresyon) 15'inin OGG-1 gen ekspresyonu düzeyleri düzelme sonrasında artmış, 6'sının azalmış olarak saptanmıştır (Şekil 15). Depresyon hastalarının OGG-1 gen ekspresyonu düzeyleri düzelme sonrasında 2,95 kat artmış olarak saptanmıştır (Z=1,999, p=0,046).



Şekil 15. Depresyon hastalarının OGG-1 geni ekspresyon düzeylerinin düzelme sonrası kat değişimi

4.4.3 OGG-1 gen ekspresyonu düzeylerinin bipolar depresyon, unipolar depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

OGG-1 mRNA ekspresyon düzeyleri ortalaması sağlıklı gönüllü grubunda 1,21 ± 0,88, unipolar depresyon grubunda 0,90 ± 0,55, bipolar depresyon grubunda 1,11 ± 0,73 olarak saptanmıştır (Şekil 16). Gruplar arasında istatistiksel anlamlılık düzeyinde düşük olarak saptanmamıştır (F=1,657, df=2, p=0,197). ANCOVA analizinde yaş, cinsiyet, beden kitle indeksi, sigara içme durumu ile düzeltilerek kontrol edildiğinde, OGG-1 düzeyleri açısından üç grup arasında fark saptanmamıştır (F=5,299, df=1, p=0,024). Düzelme dönemi OGG-1 ekspresyonu düzeyleri bipolar ve unipolar depresyon hastaları arasında farklılık göstermemektedir (Z=-1,026, p=0,305).



Şekil 16. OGG-1 geni ekspresyon düzeylerinin bipolar depresyon, unipolar depresyon hastaları ve sağlıklı gönüllüler arasında karşılaştırılması

4.5. Klinik ve biyokimyasal parametrelerin ilişkileri

Araştırmanın olgu kontrol aşamasında ilaç kullanımı olan hastalar (s=41) ve ilaç kullanımı olmayan hastalar arasında 8-OHdG/ kreatinin (s=14) düzeyleri (p=0,230), ve OGG-1 ekspresyonları (p=0,190) açısından bir fark saptanmamıştır.

Klinik ve biyokimyasal parametrelerin birbirleriyle korelasyonları Tablo 5'te sunulmuştur. 8-OHdG/ kreatinin düzeyleri ile başvuru aşamasındaki depresyon süresi arasında zayıf düzeyde negatif korelasyon (r=0,237, p=0,013); OGG-1 düzeyleri ile Algılanan Stres Ölçeği toplam skoru (r=-0,224, p=0,043) arasında zayıf düzeyde negatif korelasyon saptanmıştır. Sağlıklı bireylerde biyokimyasal parametreler ile sosyodemografik ve klinik özellikler arasında anlamlı korelasyon saptanmamıştır.

Tablo 5. Klinik ve biyokimyasal parametrelerin korelasyonları

		8-OHdG/ kreatinin	8-OHdG/ kreatinin (düzelme)	OGG-1	OGG-1 (düzelme)
8-OHdG/ kreatinin (belirtili dönem)	r	1,000	0,186	-0,018	0,265
	p		0,308	0,860	0,259
8-OHdG/ kreatinin (düzelme)	r	1,000	0,186	-0,018	0,265
	p		0,308	0,860	0,259
OGG-1 (belirtili dönem)	r	-0,018	-0,288	1,000	**-0,784****
	p	0,860	0,153		**<0,001**
OGG-1 (düzelme)	r	0,265	**0,537***	**-0,784****	1,000
	p	0,259	**0,032**	**<0,001**	
Yaş	r	0,073	-0,003	-0,025	0,065
	p	0,440	0,985	0,801	0,779
Beden kitle indeksi	r	0,059	-0,03	0,10	-0,26
	p	0,530	0,87	0,36	0,28
Depresif dönemin süresi	r	**0,237***	-0,039	0,078	-0,366
	p	**0,013**	0,833	0,443	0,103
Özkıyım girişimi sayısı	r	-0,067	0,145	-0,125	0,005
	p	0,478	0,428	0,215	0,981
Young Mani Ölçeği (toplam puan)	r	0,108	-0,112	-0,035	0,124
	p	0,266	0,542	0,738	0,592
Hamilton Depresyon Ölçeği (toplam puan)	r	0,131	-0,015	-0,082	-0,407
	p	0,176	0,936	0,428	0,067
Hipomani Soru Listesi-32 (toplam puan)	r	0,031	-0,099	0,080	-0,296
	p	0,763	0,609	0,465	0,193
Algılanan Stres Ölçeği (toplam puan)	r	-0,017	0,162	**-0,224***	0,430
	p	0,870	0,409	**0,043**	0,051
Yaşam Biçimi Ölçeği (toplam puan)	r	-0,133	0,002	0,150	-0,180
	p	0,228	0,994	0,188	0,461
r=korelasyon katsayısı, p=p değeri, (*) p<0.05					

Depresif belirtilerin düzelmesi sonrası 8-OHdG/ kreatinin düzeylerinin belirtili dönem 8-OHdG/ kreatinin düzeylerine oranı, düzelme sırasındaki OGG-1 ekspresyonu düzeyleri (p=0,036), depresif dönemin süresi (<0,001), özkıyım girişimi sayısı (0,023) değerleri ile doğrusal ilişki göstermiştir (Tablo 6).

Tablo 6. Depresif belirtilerin düzelmesi sonrası 8-OHdG/ kreatinin düzeylerinin değişimine etki eden faktörler

Bağımlı değişken: belirtilerin düzelmesi sonrası 8-OHdG/ kreatinin düzeylerinin belirtili dönemdekilere oranı	**B**	**Beta**	**t**	**p değeri**	**%95 Güven Aralığı**	
8-OHdG/ kreatinin (belirtili dönem)	-0,041	-0,134	-1,531	0,129	-0,095	0,012
OGG-1 (belirtili dönem)	-0,015	-0,049	-0,542	0,589	-0,072	0,041
OGG-1 (düzelme)	**0,040**	**0,213**	**2,128**	**0,036**	**0,003**	**0,078**
Yaş	-0,001	-0,034	-0,343	0,732	-0,006	0,004
Cinsiyet	-2,523	0,000	-0,001	1,000	-0,092	0,092
Sigara kullanımı	-0,021	-0,046	-0,501	0,618	-0,104	0,062
Beden kitle indeksi (kg/ m2)	0,007	0,137	1,355	0,178	-0,003	0,017
Depresyon tipi (unipolar/ bipolar)	-0,134	-0,196	-1,451	0,150	-0,317	0,049
Depresif dönemin süresi	-0,005	-0,226	-1,975	0,051	-0,010	0,001
Özkıyım girişimi sayısı	**0,057**	**0,224**	**2,298**	**0,023**	**0,008**	**0,107**
Antidepresan kullanımı	-0,089	-0,136	-1,216	0,226	-0,235	0,056
Duygudurum düzenleyici kullanımı	0,150	0,210	1,919	0,058	-0,005	0,304
Antipsikotik kullanımı	0,054	0,071	0,640	0,523	-0,112	0,219
Young Mani Ölçeği	-0,008	-0,061	-0,655	0,514	-0,032	0,016
Hamilton Depresyon Ölçeği	0,002	0,072	0,524	0,601	-0,004	0,007
Algılanan Stres Ölçeği	0,003	0,113	1,019	0,311	-0,003	0,010
Yaşam Biçimi Ölçeği	0,001	0,118	1,086	0,280	-0,001	0,003

Depresif belirtilerin düzelmesi sonrası OGG-1 ekspresyonu kat değişikliğine etki eden faktörlerin değerlendirmesi için kurulan doğrusal regresyon modeli; depresif dönemin süresinin (p<0,001) ve belirtili dönem OGG-1 ekspresyon düzeylerinin (p=0,010) düzelme sonrası OGG-

1 ekspresyonu kat değişikliğine etkisini göstermektedir (Tablo 7).

Tablo 7. Depresif belirtilerin düzelmesi sonrası OGG-1 ekspresyonu değişimine etki eden faktörler

Bağımlı değişken: depresif belirtilerin düzelmesi sonrası OGG-1 ekspresyonu düzeyleri	**B**	**Beta**	**t**	**p değeri**	**%95 Güven Aralığı**	
8-OHdG/ kreatinin düzelme kat oranı	**1,036**	**0,196**	**2,157**	**0,033**	**0,084**	**1,989**
Yaş	0,003	0,025	0,253	0,801	-0,023	0,030
Cinsiyet	-0,145	-0,055	-0,610	0,543	-0,615	0,326
Sigara kullanımı	0,162	0,068	0,751	0,454	-0,265	0,589
Beden kitle indeksi (kg/ m2)	-0,023	-0,084	-0,842	0,401	-0,076	0,031
Depresyon tipi (unipolar/ bipolar)	0,216	0,060	0,428	0,670	-0,786	1,218
Depresif dönemin süresi	**0,060**	**0,500**	**4,542**	**<0,001**	**0,034**	**0,086**
Özkıyım girişimi sayısı	-0,074	-0,054	-0,549	0,584	-0,340	0,192
Antidepresan kullanımı	-0,472	-0,135	-1,241	0,217	-1,226	0,282
Duygudurum düzenleyici kullanımı	-0,290	-0,077	-0,707	0,481	-1,104	0,523
Antipsikotik kullanımı	-0,635	-0,159	-1,481	0,141	-1,485	0,215
Young Mani Ölçeği	0,044	0,064	0,696	0,488	-0,082	0,171
Hamilton Depresyon Ölçeği	**-0,039**	**-0,355**	**-2,750**	**0,007**	**-0,067**	**-0,011**
Algılanan Stres Ölçeği	-0,024	-0,128	-1,315	0,191	-0,061	0,012
Yaşam Biçimi Ölçeği	0,011	0,070	0,633	0,528	-0,024	0,046

5. TARTIŞMA

Bu çalışmada DNA hasarı/onarımı ile depresif belirtiler, depresif belirtilerin şiddeti ve belirtilerin düzelmesi arasındaki ilişkilerin değerlendirilmesi amaçlanmıştır. Depresif hastalarda, sağlıklılardakilerle karşılaştırıldığında, yüksek idrar 8-OHdG/ kreatinin düzeyleri, OGG-1 gen ekspresyonu düzeylerinde değişiklikler ve depresif hastaların belirtilerinin düzelmesiyle DNA hasarı/onarımı belirteçlerinde değişiklikler saptanacağı varsayılmıştır. Depresyon hastalarının idrar 8-OHdG/ kreatinin düzeylerinin sağlıklı bireylerinkine göre yüksek, OGG-1 ekspresyon düzeylerinin sağlıklı bireylere göre düşük olduğunu gösteren bulgularımız araştırmanın hipotezlerini ve depresyon hastalarında oksidatif hasarda ve DNA hasarında artış gösteren metaanalizlerin sonuçlarını destekler niteliktedir (7,18).

5.1. Depresyonda 8-OHdG Düzeyleri

Depresif belirtiler ile oksidatif DNA hasarına odaklanan çalışmaların büyük bir kısmında DNA hasarı, DNA oksidasyonun major ürünlerinden biri olan 8-OHdG nükleozidinin kantitatif ölçümüyle değerlendirilmiştir (7). Depresyonda DNA hasarını inceleyen tek metaanaliz çalışmasında, 8-OHdG'nin depresyonda artmış olduğu, ancak araştırma için kullanılan örnek tipine ve ölçüm yöntemlerine bağlı olarak sonuçlarda farklılıklar ortaya çıkabileceği öne sürülmüştür (7).

8-OHdG onarım süreçlerinin etkisiyle DNA üzerinden kesilip ek bir yıkıma uğramaksızın idrarla atılmaktadır, kan ve diğer doku örneklerinde saptanan 8-OHdG düzeyleri süreğen onarım sürecinin etkisiyle anlık olarak değişim gösterebilirken, idrar örneklerinde tüm vücuttan atılan hasar ürünleri saptanabilmektedir (36). Öte yandan, doku örnekleri ve idrar örnekleri arasında 8-OHdG düzeyi ölçümlerinin pozitif korelasyon gösterdiğine dönük kanıtlar da sunulmuştur (37). Depresyon tanılı hastalarda saptamış olduğumuz sağlıklı bireylerinkine göre yüksek idrar 8-OHdG düzeyleri, daha önce depresyon hastalarına ait lökosit (38-42), serum (15, 43,44), plazma (45), tam kan (8,46), idrar (10,11,47-50) örneklerinde saptanmış sağlıklılarınkine göre yüksek 8-OHdG düzeyleri bulgularıyla tutarlılık göstermektedir.

5.2. Depresyonda OGG-1 Düzeyleri

Bu araştırma kapsamında 8-OHdG lezyonunun kesilip çıkarılmasından sorumlu OGG-1 enziminin gen ekspresyon düzeyleri incelenmiştir. Bulgularımız, depresyon tanısı olan

bireylerde OGG-1 gen ekspresyonunun sağlıklı bireylerdekine göre azalmış olduğunu göstermektedir. Bugüne kadarki yazında, depresyonda DNA BÇO süreçleri ile ilgili bilgi sınırlıdır. Kanser hastaları ile yapılmış iki çalışma, depresyon eş tanısına sahip olan hastaların OGG-1 ekspresyonlarının depresyonu olmayanlara göre artmış olduğunu rapor etmiştir (14,15). Bizim bulgularımız ile bu iki çalışmanın bulguları arasındaki çelişki, hasta gruplarının seçimi ile ilişkili olabilir. Bu araştırmada depresyon dışındaki tüm hastalıkların dışlanmış olması, DNA hasar onarım dinamiklerinin hali hazırda kanser tanısı olan hastalardan farklı olmasının nedeni olabilir.

Depresyon tanılı hastaların OGG-1 ekspresyon düzeylerini sağlıklılarınkiyle karşılaştıran bir başka çalışmada OGG-1 ekspresyon düzeylerinde %25'lik bir artış olduğu rapor edilmiştir (51). Bizim araştırmamızla karşılaştırıldığında daha küçük örnekleme sahip olan bu çalışmada OGG-1 ekspresyonunu etkileyebilecek yaş, cinsiyet, sigara kullanımı, beden kitle indeksi gibi faktörlere göre düzeltme yapılmamış olması sonuçlarımız arasındaki farklılığın bir nedeni olarak yorumlanabilir. Öte yandan daha güncel olarak, depresyondaki hastaların BÇO etkinliğinin sağlıklılarınkine göre daha düşük olduğu gösterilmiştir (43). Kullanılan biyokimyasal teknikler bizim çalışmamızdakilere göre farklılıklar göstermekle birlikte, benzer bir hasta popülasyonunda benzer parametrelerin ölçüldüğü bu çalışmayla bulgularımız uyumludur.

5.3. Depresif Belirtilerin Düzelmesi Sonrası 8-OHdG ve OGG-1 Düzeyleri

Depresyon belirtileri düzelen hastaların izleminde idrar 8-OHdG düzeylerinde anlamlı bir azalma saptanmıştır. Bugüne kadarki yazında, DNA hasarını depresyon hastalarının belirtili dönemleri ve düzelme sonrası arasında karşılaştıran tek çalışmada, depresyondaki bireylerin plazmalarında sağlıklılara göre daha yüksek 8-OHdG düzeylerinin olduğu, 8 haftalık antidepresan tedavisi sonrası tedaviye yanıt veren bireylerin 8-OHdG düzeylerinde anlamlı bir değişiklik saptanmadığı rapor edilmiştir (12, 45). Sözü geçen çalışmada hastalık ve düzelme dönemi karşılaştırılan hasta sayısının (s=12) düşüklüğü Tip II hata olasılığını akla getirmektedir, bizim çalışmamızdaki örneklem büyüklüğünün (s=30) daha büyük oluşu sonuçtaki farklılığın nedeni olabilir. Stres altındaki farelerde venlafaksin ile düzelme serum ve hipokampal 8-OHdG düzeylerinde düşme gösteren bir hayvan çalışması da bulgumuzu destekler niteliktedir (52).

Güncel bir başka araştırmada, yetişkin anksiyete ve/veya depresyon hastalarında 8-OHdG'nin depresyon ve antidepresan kullanımı ile ilişkisi incelenmiş, antidepresan

kullananlarda bu belirteç düşük bulunurken, hastalık veya iyilik hali 8-OHdG/ kreatinin düzeyleri ile ilişki göstermemiştir (53). Ancak bu araştırmanın, sosyal anksiyete bozukluğu, yaygın anksiyete bozukluğu, panik bozukluk, agorafobi, distimi, major depresif bozukluk gibi bir birinden nörobiyolojik olarak farklı olan ve toplumda yaygın olarak görülen çok sayıda hastalık grubunu bir araya getirmiş ve sağlıklı bireylerle karşılaştırmış olması, sonuçlarının depresif bozukluklar açısından yorumlanmasını güçleştirmektedir.

Depresyon belirtileri düzelen hastaların izleminde OGG-1 düzeylerinde ortalama 2,9 kat artış saptanmıştır. Bu bulgumuz, OGG-1 düzeylerinde depresif belirtili dönemde ortaya çıkan azalmanın geri dönüşümlü olabileceğini işaret etmektedir. Öte yandan, bipolar bozukluk ötimik döneminde OGG-1 ekspresyon düzeylerinin sağlıklı bireylerdekilere göre düşük seyrediyor olduğu yönündeki bulgular göz önünde tutulduğunda bu kat artışının geçici olabileceği düşünülebilir (16, 17). Diğer bir ihtimal, unipolar ve bipolar depresyonda düzelme ile OGG-1 ekspresyonundaki değişikliklerin farklı seyrediyor oluşudur. Ötimik dönemdeki unipolar ve bipolar bozukluk hastalarını karşılaştıran çalışmalar, bu konunun aydınlatılmasına yardımcı olabilir.

5.4. Depresyonun Klinik Özelliklerinin 8-OHdG ve OGG-1 Düzeyleri İle İlişkisi

Bu çalışmaya bipolar ve unipolar depresyon hastaları dahil edilmiştir. Bulgularımız, sosyo-demografik veriler, yaşam biçimleri, algılanan stres düzeyleri, hastalık şiddeti ve özellikleri açısından bipolar ve unipolar depresyon hastalarının benzer özellikte olduğunu göstermektedir (Tablo 2). Hastalık grupları arasında tek farklılık Hipomani Soru Listesi toplam skorları arasında saptanmıştır. Hipomani Soru Listesi hipomanik/manik belirtileri sorgulayan bir ölçüm aracı olduğu ve bipolar bozukluk tanısı için en az bir hipomanik/manik dönemin olması zorunlu olduğu için bu farklılık doğaldır. Klinik olarak değerlendirildiğinde, unipolar ve bipolar depresyon hastalarının homojen özellik göstermektedir. İki tanı arasında idrar 8-OHdG/ kreatinin ve OGG-1 mRNA ekspresyonu düzeyleri arasında bir farklılık saptanmamıştır.

8-OHdG düzeyleri unipolar ve bipolar depresyon hastaları arasında anlamlı bir farklılık göstermemektedir. Üçlü karşılaştırmalarda, unipolar depresyon hastalarının ortalama 8-OHdG düzeyi sağlıklılarınkine göre yüksek saptanırken, bipolar depresyon hastalarında görünür bir yüksekliğe rağmen anlamlı farklılık saptanamaması, bipolar depresyon grubunun sayıca daha az kişiden oluşmasına bağlı olarak Tip II hata olarak yorumlanabilir. Depresif hastalarda

saptamış olduğumuz idrar 8-OHdG/ kreatinin yüksekliği, daha önce bipolar depresyon rapor etmiş olduğumuz plazma 8-OHdG/ kreatinin yüksekliği ile tutarlılık göstermektedir (8).

OGG-1 ekspresyon düzeyleri belirtili dönemde ve düzelme sonrasında unipolar ve bipolar depresyon hastaları arasında anlamlı bir farklılık göstermemektedir. Üç grup karşılaştırmasında, sağlıklı bireyler ve depresyon hastaları arasında iki grup karşılaştırmasında saptanmış farklılığın saptanamaması Tip II hata olarak yorumlanmıştır. Bipolar bozuklukta ötimide (16,17), manik ve depresif dönemlerde (17) OGG-1 ekspresyonu düzeylerinde azalma bildirilmiştir.

Bulgularımız, depresyon hastalarında DNA hasarının artmış olduğunu, belirtilerin azalması ile hasarın azaldığını göstermektedir. Hastaların büyük kısmında DNA hasar düzeyleri belirtilerin düzelmesi ile azalırken, 6 hastada azalma saptanmamıştır. Azalma saptanmayan hastaların sayısının azlığı nedeniyle bir alt grup analizi yapmak mümkün olmamakla birlikte, doğrusal regresyon analizinin sonuçları, özkıyım girişimlerinin sayısında artışın, 8-OHdG düzeylerinde düzelmenin 8-OHdG düzeylerine etkisini azalttığı şeklinde yorumlanabilir. Özkıyım girişimi sayısının yüksekliği hastalığın tekrarlayıcı ya da kronik özelliklerini işaret ediyor olabilir ve biyolojik düzelmenin ortaya çıkmaması bu hastaların özel bir grup oluşturmasına bağlı olabilir.

Bulgularımız, depresyon hastalarında OGG-1 düzeylerinin azaldığını, belirtilerin iyileşmesiyle OGG-1 düzeylerinin arttığını göstermektedir. Doğrusal regresyon analizi sonuçlarına göre, düzelme dönemindeki OGG-1 ekspresyon düzeylerinin depresyonun süresi ile artış göstermektedir. Bu bulgu, hasarı kompanse etmek üzere gen ekspresyonunun zaman içinde up-regüle olduğunu düşündürebilir. Ancak 6 olguda artış saptanmamıştır. Bazal depresyon şiddeti ile düzelme dönemindeki OGG-1 düzeylerinin azalma eğiliminde oluşu, şiddetli depresyonda up-regülasyonun geciktiğini, ya da bu sürecin bozulduğu şeklinde yorumlanabilir. Bu bulgular, OGG-1 düzeylerindeki artış depresyon süresince yüksek seyreden 8-OHdG düzeylerine yanıt olarak ortaya çıkmış bir up-regülasyonu ifade ediyor olabileceği yönünde yorumlanmıştır.

5.5. Yaşam Biçimi Özelliklerinin 8-OHdG ve OGG-1 Düzeyleri İle İlişkisi

Bulgularımız depresif hastaların beslenme, fiziksel aktivite, kişiler arası ilişkiler gibi yaşam biçimi ile ilgili parametreler açısından sağlıklı bireylerden daha kötü durumda olduğunu göstermektedir. Yaşam ve beslenme biçimlerinin DNA hasarına etkilerini inceleyen

araştırmalar, sigara ve/veya alkol kullanımlarının, olumsuz beslenme alışkanlıklarının, ağır koşullarda çalışmanın ve kronik stresin DNA hasarında artışla, günlük hafif egzersizin, sağlıklı beslenmenin DNA hasarında azalma ile ilişkisini destekler veriler sunmaktadır (54-60). DNA hasarı ve onarımı değişikliklerinin depresyonun bir özelliği olup olmadığının değerlendirilebilmesi için, bu değişikliklere yaşam biçimi ile ilişkili faktörlerin katkısının değerlendirilmesi gerekmektedir. Bu nedenle, DNA hasar ve onarım süreçlerini inceleyen çalışmalarda, yaşam biçimine ilişkili faktörlerin göz önünde tutulması önerilmiştir (8).

Bu bilgiler göz önünde tutularak, araştırmanın olgu kontrol aşamasındaki karşılaştırmalarda yaş, cinsiyet, sigara kullanımı ve beden kitle indeksine göre istatistiksel düzeltmeler yapılmıştır. Hasta ve sağlıklı gruplarında yaşam biçimi (beslenme, egzersiz alışkanlıkları, stres yönetimi vb.), mesleki ve ailesel stresörler, algılanan stres düzeyi ölçümleri yapılmıştır. Hastaların v e sağlıklı bireylerin, yaşam biçimi ve algılanan stres ölçümleri ile DNA hasarı ve onarımı parametreleri arasında anlamlı bir ilişki saptanmamıştır. Ancak oksidatif DNA hasarı ve onarımı belirteçleri ile yapılan korelasyon analizlerinde sadece bazal OGG-1 değerleri algılanan stres değerleri ile zayıf bir doğrusal ilişki göstermiş olsa da, bu depresif dönem hastalarındaki yüksek algılanan stres düzeylerinden kaynaklandığı düşünülmüştür. Regresyon analizlerinde yaşam biçimi ve algılanan stres parametreleri ile DNA hasarı ve onarımı belirteçleri arasında ilişki saptanmamıştır.

5.6. Araştırmanın Güçlü Yanları ve Kısıtlılıkları

Bildiğimiz kadarıyla bu çalışma, ek tanısı olmaksızın klinik depresyon tanısı almış hastalarda DNA hasar ve onarım süreçlerini bir arada çalışan ilk çalışmadır. DNA hasar ve onarım süreçlerini bir arada çalışılması, her iki süreç için en önde gelen belirteçlerin kullanılması, belirteçlerin ölçümünde güvenilir yöntemlerin kullanılmış olması, depresif bireylerin boylamasına izleminin bulunması, yaşam biçimine ilişkin faktörlerin göz önünde tutulması bu araştırmanın güçlü yanlarıdır.

Araştırmamızda DNA hasarı en yaygın kullanılan hasar belirteci olan 8-OHdG ile ölçülmüştür. Kromatografik yöntemler DNA hasar ölçümleri için standart ölçümler olarak kabul edilmektedir, LC-MS-MS ölçümlerinin yapılmış olması araştırmanın teknik açıdan güçlü yanlarından biridir (7,36). Dokularda DNA hasar belirteci ölçümlerinde onarım süreçlerinin etkisi dışlanamaktadır, üstelik tek dokuda (lökosit, plazma vb.) hasar ölçümü tüm vücuttaki oksidatif yükü tam olarak yansıtmamaktadır (36). Bu nedenle, idrar örneklerinde çalışılmış

olması bu araştırmanın güçlü yanlarından biridir. İdrar örnekleri ile çalışılırken olası problem, idrardaki metabolit konsantrasyonunun glomerüler filtrasyon hızından etkilenmesidir (36). Araştırmamızda, idrar 8-OHdG düzeylerinin daha önceki çalışmaların önerdiği şekilde kreatinin düzeylerine göre düzeltilmiş olması da bu araştırmanın güçlü bir yanıdır. Araştırmanın izlem aşamasının bulunması yaşam biçimine bağlı karıştırıcı faktörlerin kontrol edilerek belirtilerin ve düzelmenin etkisini değerlendirilmesine olanak sağlamıştır.

Araştırmamızda bütçe sınırı nedeniyle baz çıkarma onarımında rol alan enzimler arasında 8-OHdG hasarına özgül enzim olan, ve baz çıkarma onarımını başlatan enzim olan OGG-1 incelenmek üzere seçilmiştir. Baz çıkarma yolağındaki diğer genlerin de inceleneceği ve OGG-1 enzimini farklı örneklerde protein ve enzim aktivitesi düzeyinde inceleyen yeni araştırmalar bu konudaki bilgi birikimine katkı sunacaktır. İkinci kısıtlılık araştırmanın hastaların ilaç kullanımlarının etkisini değerlendirecek bir desene sahip olmayışıdır. Psikotrop ilaçların DNA hasar ve onarım süreçlerine etkilerde bulunduğuna dönük kanıtlar mevcuttur (52,61-63). Ancak araştırmamızda depresyon hastalarının bazal ölçümleri üzerinden yürüttüğümüz analizde ilaç kullanımları ile bir ilişki saptanmamıştır. Düzelme dönemini izleyebildiğimiz hasta sayısı bazı alt grup analizlerinde Tip II hata riskini ortaya çıkarmıştır.

6. SONUÇ

Depresyon hastalarında sağlıklılarla karşılaştırıldığında daha fazla oksidatif DNA hasarı ve daha düşük DNA onarım geni ekspresyonu saptanmıştır. Bulgularımız depresyonda akut hastalık döneminde sistemik oksidatif hasarın arttığı ve onarım mekanizmasının ise yetersiz olduğu yönünde yorumlanabilir. Depresif bireylerin belirtilerinin düzelmesiyle 8-OHdG düzeylerinin azalması ve OGG-1 ekspresyon düzeylerinin artması, DNA hasar lezyonlarının DNA onarım sistemindeki geri dönüşümlü bozulmaların sonucu olabileceğini düşündürmektedir. Verilerimiz, DNA hasar/onarım süreçlerinin depresif dönemlerin nörobiyolojisi ile ilişkili olabileceğini göstermektedir. DNA hasarı ve onarımı ile ilgili yeni çalışmalar depresyonun nörobiyolojisinin daha iyi anlaşılmasına katkıda bulunabilir.

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EK 1: Etik Kurul Onayı

KLİNİK ARAŞTIRMALAR ETİK KURULU KARAR FORMU

ARAŞTIRMANIN AÇIK ADI	Depresyonda DNA Hasarının ve Onarımının Belirtili Dönem ve Düzelme ile İlişkisi
VARSA ARAŞTIRMANIN PROTOKOL KODU	
ETİK KURUL PROTOKOL NUMARASI	307 -SBKAEK

KARAR BİLGİLERİ	Karar No:2016/04-01	Tarih:25.02.2016
	Yukarıda bilgileri verilen başvuru dosyası ile ilgili belgeler araştırmanın/çalışmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve uygun bulunmuş olup araştırmanın/çalışmanın başvuru dosyasında belirtilen merkezlerde gerçekleştirilmesinde etik ve bilimsel sakınca bulunmadığına toplantıya katılan etik kurul üye tam sayısının salt çoğunluğu ile karar verilmiştir.	

KLİNİK ARAŞTIRMALAR ETİK KURULU	
ETİK KURULUN ÇALIŞMA ESASI	İlaç ve Biyolojik Ürünlerin Klinik Araştırmaları Hakkında Yönetmelik, İyi Klinik Uygulamaları Kılavuzu
BAŞKANIN UNVANI / ADI / SOYADI:	Prof.Dr.Ayşegül Yıldız

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		Araştırma ile ilişki		Katılım *		İmza
Prof.Dr.Ayşegül YILDIZ	Psikiyatri	DEU Tıp Fakültesi Psikiyatri Anabilim Dalı	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	katılmadı
Prof.Dr.Hülya ELLİDOKUZ	Halk Sağlığı	DEU Onkoloji Enstitüsü Prevantif Onkoloji A.D	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Prof.Dr.Nuray DUMAN	Çocuk Sağlığı ve Hastalıkları (Yeni Doğan)	DEU Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Anabilim Dalı	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Prof.Dr.Hale ÖREN	Çocuk Sağlığı ve Hastalıkları (Çocuk Hematoloji)	DEU Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Anabilim Dalı	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Prof.Dr.A.Necati GÖKMEN	Anesteziyoloji ve Reanimasyon	Anesteziyoloji ve Reanimasyon A.D	E ☒	K ☐	E ☐	H ☒	E ☐	H ☐	katılmadı
Prof.Dr.Taner DAĞCI	Fizyoloji	Ege Üniversitesi Tıp Fakültesi	E ☒	K ☐	E ☐	H ☒	E ☐	H ☐	
Doç.Dr.Pembe KESKİNOĞLU	Biyoistatistik	DEU Tıp Fakültesi Biyoistatistik ve Tıbbi Bilişim A.D	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Doç.Dr.Erdem YAKA	Nöroloji	DEU Tıp Fakültesi Nöroloji A.D	E ☒	K ☐	E ☐	H ☒	E ☐	H ☐	
Doç.Dr.Uğur Önsel TÜRK	Kardiyoloji	Ege Üniversitesi İlaç ve Farmakokinetik Arş-Uyg.Merk	E ☒	K ☐	E ☐	H ☒	E ☐	H ☐	
Doç.Dr.Yasemin BASKIN	Temel Onkoloji	DEU Onkoloji Enstitüsü Temel Onkoloji A.D	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Yard.Doç.Dr.Yasemin ERAÇ	Farmakoloji	Ege Üniversitesi Eczacılık Fakültesi Farmakoloji Anabilim Dalı	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Av.Semra MARMARA	Hukuk	DEÜ Rektörlüğü	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	katılmadı
Av.Nazan PEDÜKCOŞKUN	Hukuk	Alsancak Nevvar Salih İşgören Hastanesi	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	
Hayat ALBAYRAK	Emekli	Sağlık Mesleği Mensubu Olmayan Üye	E ☐	K ☒	E ☐	H ☒	E ☐	H ☐	

*:Toplantıda Bulunma

Etik Kurul Başkanının
Unvanı/Adı/Soyadı: Prof.Dr.Ayşegül Yıldız
İmza:

Not: Etik kurul başkanı, imzasının yer almadığı her sayfaya imza atmalıdır.

EK 2: Genetik çalışma -Hastalar İçin Bilgilendirilmiş Olur Formu

Ruhsal bozuklukların ortaya çıkmasına yol açan etmenler henüz tam anlaşılamamıştır. Ancak bazı genetik ve çevresel etmenlerin rol aldığı bilinmektedir. Bu çalışmada yapılacak analizlerle inflamasyon belirteçleri, oksidatif hücre hasarı ve onarımını araştırmak üzere 10 cc kan ve sabah ilk idrar örneğiniz alınacaktır. Bu araştırma için toplam 160 gönüllüden kan alınması planlanmaktadır.

Bu çalışma kapsamında size herhangi bir ilaç uygulaması yapılmayacak, görmekte olduğunuz tedavi değiştirilmeyecektir. Bu araştırma için öncelikle sizinle araştırmacı tarafından bir saatlik tanısal bir görüşme yapılacaktır. Bu görüşme sırasında hastalığınızın şiddetinin ve özelliklerinin belirlenmesine yönelik bir dizi ölçek uygulanacaktır. Daha sonra aynı hafta içerisinde sabah 09.00-10.00 arasında sabah ilk idrarınızı ve kan örneğinizi vermek üzere hastaneye davet edileceksiniz. Bu işlemin kan alınması dışında sizin üzerinizde hiçbir etkisi olmayacaktır. Kan alınması sırasında en sık görülen yan etkiler, kolunuzda iğne yerinde ağrı ve morarmadır.

İki ay sonra klinik değerlendirmeleriniz, kan ve idrar tahlilleriniz yeniden yapılacaktır. Eğer 2 ayın sonunda hastalığınızda yeterli derecede düzelme saptanmazsa, 3. ayda sizinle tekrar görüşülecek, kan ve idrar örnekleriniz tekrar alınacaktır.

Yapılan çalışmanın sonuçları şu anda size veya ailenize bir tanı veya tedavi hizmeti vermeyebilir, ancak uzun vadede hastalığınızla ilgili elde edilebilecek sonuçlar etkilenmiş kişi ve ailelere yeni tedavi yöntemleri ve koruyucu önlemlerin geliştirilmesi amaçlı yarar sağlayabilecektir.

Bu araştırmanın sonuçları yalnızca bilimsel amaçlarla kullanılacak, veriler ve size ait bilgiler gizli tutulacaktır. Yerel Etik Kurullar'ın ve T.C. Sağlık Bakanlığı'nın gerek gördüğü durumlarda kayıtlarınız bu kurumlara açık olacaktır. Bu formun imzalanmasıyla bu erişime izin vermiş olacaksınız. Kimliğinizi ortaya çıkaracak kayıtlar gizli tutulacak, kamuoyuna açıklanmayacaktır. Araştırma sonuçlarının yayımlanması halinde dahi kimliğiniz gizli kalacaktır.

Araştırma konusuyla ilgili ve sizin araştırmaya devam etme isteğinizi etkileyebilecek yeni bilgiler elde edildiğinde zamanında bilgilendirileceksiniz. Araştırmayla ilgili herhangi bir sorunuzu veya sorununuzu 02324124151 numaralı telefondan doktorunuz Deniz Ceylan'a danışabilirsiniz.

Bu çalışma sırasında uygulanacak testlerin ve araştırma ile ilgili gerçekleştirilecek diğer

işlemlerin masrafları size veya güvencesi altında bulunduğunuz resmi ya da özel hiçbir kurum veya kuruluşa ödetilmeyecektir.

Bu çalışmanın tümüne ya da bir kısmına katılmayı reddetme ya da araştırma başladıktan sonra devam etmeme hakkına sahipsiniz. Bu çalışmaya katılmanız veya başladıktan sonra herhangi bir aşamasında ayrılmanız daha sonraki tıbbi bakımınızı etkilemeyecektir. Araştırmacı da gönüllünün kendi rızasına bakmadan, gereklilik ortaya çıkarsa olguyu araştırma dışı bırakabilir.

Yukarıda yer alan ve araştırmadan önce gönüllüye verilmesi gereken bilgileri gösteren Bilgilendirilmiş Onam Formunu okudum ya da bana okunmasını sağladım. Bu bilgilerin içeriği ve anlamı, yazılı ve sözlü olarak açıklandı. Aklıma gelen bütün soruları sorma olanağı tanındı ve sorularıma yeterli cevaplar aldım. Çalışmaya katılmadığım ya da katıldıktan sonra çekildiğim durumda, hiçbir yasal hakkımdan vazgeçmiş olmayacağım. Bu koşullarla, söz konusu araştırmaya hiçbir baskı ve zorlama olmaksızın gönüllü olarak katılmayı kabul ediyorum. *Bu formun imzalı bir kopyası bana verildi.*

“Depresyonda DNA hasarının ve onarımının belirtili dönem ve düzelme ile ilişkisi” araştırması kapsamında alınan biyolojik örneklerimin (kan);

- Sadece yukarıda bahsi geçen araştırmada kullanılmasına izin veriyorum
- İleride yapılması planlanan tüm diğer araştırmalarda kullanılmasına izin veriyorum
- Hiçbir koşulda kullanılmasına izin vermiyorum

Hastanın;

Adı:……………………Soyadı:………………...Tarih:……………Telefon: …………..

İmza :………..

Hastanın veli ya da vasisi;

Adı :……………………Soyadı :………………...Tarih :……………Telefon:…………

İmza :………..

Olur Alma İşlemine Başından Sonuna Kadar Tanıklık Eden Kuruluş Görevlisinin

Adı :……………………Soyadı :………………...Tarih :……………Telefon …………

İmza :………..

Araştırma Yapan Araştırmacının

Adı:……………………Soyadı:………………...Tarih :……………Telefon …………..

İmza :………..

EK 3: Genetik çalışma-Sağlıklı Gönüllüler İçin Bilgilendirilmiş Olur Formu

Ruhsal bozuklukların ortaya çıkmasına yol açan etmenler henüz tam anlaşılamamıştır. Ancak bazı genetik ve çevresel etmenlerin rol aldığı bilinmektedir. Bu çalışmada yapılacak analizlerle inflamasyon belirteçleri, oksidatif hücre hasarı ve onarımını araştırmak üzere 10 cc kan ve sabah ilk idrar örneğiniz alınacaktır. Bu araştırma için toplam 160 gönüllüden kan alınması planlanmaktadır.

Bu çalışmada size herhangi bir ilaç verilmeyecek, kan ve idrar alımı dışında hiçbir ek işlem yapılmayacaktır. Bu araştırma için öncelikle doktorunuz sizinle bir saatlik tanısal bir görüşme yapacaktır. Daha sonra aynı hafta içerisinde sabah 09.00-10.00 arasında sabah ilk idrarınızı ve kan örneğinizi vermek üzere hastaneye davet edileceksiniz. Bu işlemin kan alınması dışında sizin üzerinizde hiçbir etkisi olmayacaktır. Kan alınması sırasında en sık görülen yan etkiler, kolunuzda iğne yerinde ağrı ve morarmadır.

Yapılan çalışmanın sonuçları şu anda size veya ailenize bir tanı veya tedavi hizmeti vermeyebilir, ancak uzun vadede hastalığınızla ilgili elde edilebilecek sonuçlar etkilenmiş kişi ve ailelere yeni tedavi yöntemleri ve koruyucu önlemlerin geliştirilmesi amaçlı yarar sağlayabilecektir.

Bu araştırmanın sonuçları yalnızca bilimsel amaçlarla kullanılacak, veriler ve size ait bilgiler gizli tutulacaktır. Yerel Etik Kurullar'ın ve T.C. Sağlık Bakanlığı'nın gerek gördüğü durumlarda kayıtlarınız bu kurumlara açık olacaktır. Bu formun imzalanmasıyla bu erişime izin vermiş olacaksınız. Kimliğinizi ortaya çıkaracak kayıtlar gizli tutulacak, kamuoyuna açıklanmayacaktır. Araştırma sonuçlarının yayımlanması halinde dahi kimliğiniz gizli kalacaktır.

Araştırma konusuyla ilgili ve sizin araştırmaya devam etme isteğinizi etkileyebilecek yeni bilgiler elde edildiğinde zamanında bilgilendirileceksiniz. Araştırmayla ilgili herhangi bir sorunuzu veya sorununuzu 02324124151 numaralı telefondan doktorunuz Deniz Ceylan'a danışabilirsiniz.

Bu çalışma sırasında uygulanacak testlerin ve araştırma ile ilgili gerçekleştirilecek diğer işlemlerin masrafları size veya güvencesi altında bulunduğunuz resmi ya da özel hiçbir kurum veya kuruluşa ödetilmeyecektir.

Bu çalışmanın tümüne ya da bir kısmına katılmayı reddetme ya da araştırma başladıktan sonra devam etmeme hakkına sahipsiniz. Bu çalışmaya katılmanız veya başladıktan sonra herhangi bir safhasında ayrılmanız daha sonraki tıbbi bakımınızı etkilemeyecektir. Araştırmacı

da gönüllünün kendi rızasına bakmadan, gereklilik ortaya çıkarsa olguyu araştırma dışı bırakabilir.

Yukarıda yer alan ve araştırmadan önce gönüllüye verilmesi gereken bilgileri gösteren Bilgilendirilmiş Onam Formunu okudum ya da bana okunmasını sağladım. Bu bilgilerin içeriği ve anlamı, yazılı ve sözlü olarak açıklandı. Aklıma gelen bütün soruları sorma olanağı tanındı ve sorularıma yeterli cevaplar aldım. Çalışmaya katılmadığım ya da katıldıktan sonra çekildiğim durumda, hiçbir yasal hakkımdan vazgeçmiş olmayacağım. Bu koşullarla, söz konusu araştırmaya hiçbir baskı ve zorlama olmaksızın gönüllü olarak katılmayı kabul ediyorum.

Bu formun imzalı bir kopyası bana verildi.

"Depresyonda DNA hasarının ve onarımının belirtili dönem ve düzelme ile ilişkisi" araştırması kapsamında alınan biyolojik örneklerimin (kan);

- Sadece yukarıda bahsi geçen araştırmada kullanılmasına izin veriyorum
- İleride yapılması planlanan tüm diğer araştırmalarda kullanılmasına izin veriyorum
- Hiçbir koşulda kullanılmasına izin vermiyorum

Sağlıklı gönüllünün

Adı:…………………….Soyadı:……………………Tarih: Telefon no:

İmza:

Olur Alma İşlemine Başından Sonuna Kadar Tanıklık Eden Kuruluş Görevlisinin

Adı:……………………Soyadı:…………………Tarih: İmza:

Araştırma Yapan Araştırmacının

Adı:…………………………..Soyadı:…………………..Tel:………… İmza:

EK 4: ÖZGEÇMİŞ

DENİZ CEYLAN TUFAN ÖZALP

Kişisel Bilgiler

İletişim Bilgileri

Kimlik Numarası	26356286806
Doğum Tarihi	08/02/1982
İletişim Adresi	Izmir Ekonomi Üniversitesi, Sağlık Myo, Sakarya Cad. No:156
Telefon	(532) 501 49 62
E-posta	denizceylandr@gmail.com
Web Adresi	

Eğitim Bilgileri

01 Ağustos 2009 - 22 Nisan 2014 (4 yıl 9 ay)
Tıpta Uzmanlık, Anadal/Normal Öğretim, DOKUZ EYLÜL ÜNİVERSİTESİ, TÜRKİYE
TIP FAKÜLTESİ, DAHİLİ TIP BİLİMLERİ BÖLÜMÜ
Tez Başlığı: Bipolar Bozuklukta Oksidatif Dna Hasarı, Onarımı Ve Oksidatif Hasarın Nörotrofik Faktörlerle Ilişkisi
Tez Konusu: Bipolar Bozukluk
Tarih: 14 Nisan 2014
Tez Danışmanı: AYŞEGÜL ÖZERDEM
Diploma Numarası: 110116

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TIP FAKÜLTESİ, TIP PR.
Diploma Numarası: 12386
Ağırlıklı Genel Not Ortalaması: 69.28 / 100.0

Deneyim / İşyeri Bilgileri

01 Temmuz 2017 - Şu Anda (1 yıl 8 ay) (Tam Zamanlı)
DOKTORALI ÖĞRETİM ÜYESİ, YRD. DOÇ. DR., İZMİR EKONOMİ ÜNİVERSİTESİ

01 Temmuz 2014 - 01 Nisan 2017 (2 yıl 10 ay) (Tam Zamanlı)
UZMAN (DR.), DİĞER (Gümüşhane devlet Hastanesi)

01 Nisan 2014 - 01 Temmuz 2014 (4 ay) (Tam Zamanlı)
UZMAN (DR.), DR., DİĞER (Dokuz Eylül Üniversitesi)

01 Ocak 2009 - 01 Nisan 2014 (5 yıl 4 ay) (Tam Zamanlı)
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01 Kasım 2012 - 01 Nisan 2013 (6 ay) (Tam Zamanlı)
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Yabancı Dil Bilgileri

İNGİLİZCE (Okuma: İyi, Yazma: İyi, Konuşma: İyi)

ALMANCA (Okuma: Başlangıç, Yazma: Başlangıç, Konuşma: Başlangıç)

Bilimsel Teknolojik Faaliyet Alanları

Bilimsel Teknolojik Faaliyet Alanı Bilgileri Sağlık Bilimleri -- Tıp -- Dahili Tıp Bilimleri -- Psikiyatri

Anahtar Kelimeler

Bipolar Bozukluk

Depresyon

DNA Hasarı

Mitokondriyal Disfonksiyon

Bilişsel Sinirbilim

Oksidatif Stres

İnflamasyon

Duygudurum Bozuklukları

Psikotik Bozukluklar

Psikoz

İnflamazom

Mikro RNA

Uzaktan Hasta Takibi

HPA Ekseni

Çevresel Stres

Kadın Sağlığı

Erken Klinik Tanı

Nörosteroid

N-Metil-D-aspartat Reseptörü

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TÜBİTAK Burs ve Destekleri

Proje Bilgileri

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Oluşturma Zamanı: 21 Şubat 2019 06:45:19

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BİDEB Destekleri

Panelistlik/İzleyicilik/Raportörlük Sayısı

Panelistlik/Dış Danışmanlık Sayısı	ARDEB/BİDEB 0	TEYDEB 0	Toplam 0
İzleyicilik/Danışmanlık Sayısı	ARDEB/BİDEB 0	TEYDEB 0	Toplam 0
Raportörlük Sayısı	ARDEB/BİDEB 0	TEYDEB 0	Toplam 0

Oluşturma Zamanı: 21 Şubat 2019 06:45:19

Journal of Affective Disorders 166 (2014) 193–200

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

Alterations in BDNF (brain derived neurotrophic factor) and GDNF (glial cell line-derived neurotrophic factor) serum levels in bipolar disorder: The role of lithium

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ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form
8 May 2014
Accepted 9 May 2014
Available online 22 May 2014

Keywords:
BDNF (Brain-derived neurotrophic factor)
GDNF (glial cell line-derived neurotrophic factor)
Bipolar disorder
Lithium
Valproate

ABSTRACT

Objective: Brain-derived neurotrophic factor (BDNF) has been consistently reported to be decreased in mania or depression in bipolar disorders. Evidence suggests that Glial cell line-derived neurotrophic factor (GDNF) has a role in the pathogenesis of mood disorders. Whether GDNF and BDNF act in the same way across different episodes in bipolar disorders is unclear.
Method: BDNF and GDNF serum levels were measured simultaneously by enzyme-linked immunosorbent assay (ELISA) method in 96 patients diagnosed with bipolar disorder according to DSM-IV (37 euthymic, 33 manic, 26 depressed) in comparison to 61 healthy volunteers. SCID- I and SCID-non patient version were used for clinical evaluation of the patients and healthy volunteers respectively. Correlations between the two trophic factor levels, and medication dose, duration and serum levels of lithium or valproate were studied across different episodes of illness.
Results: Patients had significantly lower BDNF levels during mania and depression compared to euthymic patients and healthy controls. GDNF levels were not distinctive. However GDNF/BDNF ratio was higher in manic state compared to euthymia and healthy controls. Significant negative correlation was observed between BDNF and GDNF levels in euthymic patients. While BDNF levels correlated positively, GDNF levels correlated negatively with lithium levels. Regression analysis confirmed that lithium levels predicted only GDNF levels positively in mania, and negatively in euthymia.
Limitations: Small sample size in different episodes and drug-free patients was the limitation of thestudy.
Conclusion: Current data suggests that lithium exerts its therapeutic action by an inverse effect on BDNF and GDNF levels, possibly by up-regulating BDNF and down-regulating GDNF to achieve euthymia.
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1. Introduction

The precise neurobiology underlying bipolar disorder (BD) is unknown. Evidence suggests disturbance in neuronal functions and neuroplasticity in the pathogenesis of the illness (Grande et al., 2010; Hashimoto, 2010; Kapczinski et al., 2008; Post, 2007). BDNF (Brain Derived Neurotrophic Factor), the most widely studied trophic factor is a key mediator for synaptic efficacy, neuronal connectivity and neuroplasticity, and the regulation of neuronal survival and control the activity of many neurotransmitter systems (Cotman and Berchtold, 2002; Duman et al., 2000). It was consistently shown to be decreased during manic, depressive (Cunha et al., 2006; Fernandes et al., 2011; Kapczinski et al., 2008; Lin, 2009; Machado-Vieira et al., 2007; Palomino et al., 2006; Tramontina et al., 2009) and even in euthymic states (Kauer-Sant'anna et al., 2008; Lin, 2009; Monteleone et al., 2008) of bipolar disorder. Glial cells have an important role in providing trophic support to neuron's metabolism and the formation of the synapsis as the third-partner in synaptic transmission (tripartite synapse) (Araque et al., 1999; Sawada et al., 2000). Glial pathology has been repeatedly reported in bipolar disorders. Reduction in numbers or density of glial cells (Brauch et al., 2006; Öngür et al., 1998; Rajkowska, 2000; Uranova et al., 2004) and alteration of related biochemical markers (Cotter et al., 2001; Machado-Vieira et al., 2002; Öngür et al., 2008; Webster et al., 2005) have been reported. Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β super family which

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http://dx.doi.org/10.1016/j.jad.2014.05.012
0165-0327/© 2014 Published by Elsevier B.V.

binds to the GDNF-family receptor α1 (GFRα1) (Airaksinen and Saarma, 2002), and influences the development, survival, and differentiation of neurons, increases the density and neurite length (Ducray et al., 2006). Findings related to the peripheral measures of GDNF are inconsistent. Increased GDNF serum immunocontents in mania and depression (Rosa et al., 2006), plasma levels in euthymia (Barbosa et al., 2011), decreased whole blood levels in manic or depressive episodes (Zhang et al., 2010) and in euthymia (Takebayashi et al., 2005) were reported.

Neurons and glial cells have a bidirectional communication at the synapses to regulate synaptic plasticity (Allen and Barres, 2005; Bezzi and Volterra, 2001; Todd et al., 2006). BDNF and GDNF were shown to act synergistically on survival of the injured motor neurons in experimental studies (Giehl et al., 1998; Sharma, 2006, Zurn et al., 1996). However, it is not known whether they control the illness states in bipolar disorder.

The two mood stabilizers lithium and valproate were demonstrated to up-regulate BDNF (Frey et al., 2006; Fukumoto et al., 2001; Manji et al., 2000; Quiroz et al., 2010), but the evidence related to GDNF expression are contradictory (Angelucci et al., 2003; Fukumoto et al., 2001; Su et al., 2009; Qu et al., 2011). It is also not known whether BDNF and GDNF act in a similar way in achieving euthymia. To answer these questions we measured concomitant BDNF and GDNF serum levels during illness state (manic or depressive episodes) and in euthymia in patients with bipolar disorder.

2. Method

2.1. Participants

The study was approved by the Dokuz Eylul University Hospital Ethics Committee. Ninety six Bipolar Disorder type I ($n=92$) or type II ($n=4$) (37 euthymic, 33 manic, 26 depressed) inpatients or outpatients were recruited from the Psychiatry Clinics and Bipolar Disorder Unit of the University Hospital. Bipolar Disorder diagnosis was confirmed using the Structured Clinical Interview for DSM-IV Axis I (SCID-I) (First et al., 2002) by formally trained psychiatrists for SCID. Patients were included if they fulfilled criteria for current manic and depressive episode, or currently in remission. Mixed and hypomanic episodes were excluded. Overall severity of illness, and manic or depressive episodes were assessed using the Clinical Global Impression(CGI) scale (Busner and Targum, 2007), Young Mania Rating Scale (YMRS) (Young et al., 1978) or 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) respectively. Early-onset was accepted ≤ 19 years of age (Sachs et al., 2000). Patients had no active medical conditions and were not on any medication other than their prescribed psychotrophic drugs. Eight patients (8.3%) were drug-free. Fifty-nine out of 96 patients (61.5%) were on lithium either as monotherapy ($n=8$, 4.5%) or in combination with other medications. The rest ($n=29$; 31.2%) were on non-lithium antibipolar treatment. Fifty-three patients were on valproate either as monotherapy ($n=10$, 5.6%) or in combination with other medications ($n=43$, 29.7%).

Sixty-one healthy volunteers, among the residents, faculty or staff of the university hospital, who consented to participating were included in the study. They were not on any medication and had no history of any psychiatric disorders as assessed by the SCID-I non-patient version. Written informed consents were provided from all patients and healthy volunteers.

2.2. BDNF and GDNF measurements

BDNF and GDNF measurements for each participant were performed in duplicates by investigators who were blind to the participants' state (i.e. eutyhmic, depressed, manic or healthy control). Blood samples were drawn on the same day of the psychiatric assessments.

Ten milliliters of blood were withdrawn from each participant by venipuncture into a free-anticoagulant vacuum tube. The blood was immediately centrifuged at $3000 \times g$ for 10 min, and serum was kept frozen at -80 °C until assayed. BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (ChemiKine, Millipore). Firstly, the diluted samples and BDNF standards were added and incubated overnight. Then the plates were successively incubated with biotinylated BDNF antibody at room temperature for 2 h, washed, and incubated with streptavidin conjugated to horseradish peroxidase for 1 h at room temperature. At the final stage, the plates were incubated with peroxidase substrate, tetramethylbenzidine solution. Then reaction was stopped by HCl and absorbance of the resulting yellow-colored product is measured at 450 nm (Synergy HT, BioTek, U.S.A.) to determine BDNF values that are expressed as pg/mL. The range of detection was from7.82 pg/mL to 500 pg/mL. Intra and inter assay coefficients of variations were 3.5% and 1.18%, respectively.

Total GDNF levels were also analyzed by commercial ELISA kit (Promega, USA). Before proceeding with the ELISA protocol, all sera samples were incubated with 125 uL 1 N HCl per milliliter of undiluted sera for 25–30 min at room temperature. Following neutralization with same amount NaOH, the samples were used for quantification of the total (free and conjugated) GDNF levels. According to the manufacture procedure, 96-cell plates were coated with anti-GDNF monoclonal antibody with incubation during overnight. The captured GDNF in sera samples and standard was bound by a second, specific polyclonal antibody. After washing, the amount of specifically bound antibody was then detected using a species-specific antibody conjugated to horseradish peroxidase as a tertiary reactant. The unbound conjugate was removed by washing and following incubation with a chromogenic substrate, the color change was measured at 450 nm. The amount of GDNF in the solutions was proportional to the color generated in the oxidation–reduction reaction. The between run precision was 1.43% CV.

2.3. Statistical analysis

Statistical analyses were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). Difference between dichotomous variables was assessed with χ^2 test. Normality was tested by Shapiro–Wilk test. Independent t-test was used to compare BDNF and GDNF levels between genders, early and late onset, and presence or absence of mood stabilizers (lithium and valproic acid) in patients. Continuous clinical (age, age of illness onset, total number of episodes, total illness duration, duration of last episode, doses and duration of lithium or valproate treatment) and biochemical (serum levels of BDNF, GDNF, lithium and valproat, and GDNF/BDNF ratio) variables were compared among four group's (healthy volunteers and patients in manic, depressive and euthymic states) by using One way ANOVA. Post-hoc Dunnett's $T3$ test was used for pair-wise comparisons. Pearson correlation test as indicated "r" or Spearman' rank correlation test as indicated "ρ" (if the sample size did not satisfy the parametric tests) were performed to render associations between continuous variables. Regression analyses were completed to assess the predictive effect of serum levels and duration of lithium treatment (independent variables) on the levels of BDNF and GDNF (dependent variables) across different states of illness (euthymia, mania and depression). Data are presented as means and standard deviation (S.D.) or ratios where needed. All analyses were two-sided. Significance level was accepted as $a < 0.05$.

Table 1
Demographic and clinical characteristics of the patients and controls.

	Healthy volunteer ($n=61$)	Euthymia ($n=37$)	Mania ($n=33$)	Depression ($n=26$)	F/χ^2	df	p values	Pair wise comparisons	p values
Age (years; Mean ± SD)	38.29 ± 11.59	36.24 ± 10.02	36.77 ± 12.02	42.54 ± 10.52	1.975	3	0.120		
Gender (female %)	56.7	62.2	65.7	32.1	8.373[a]	3	0.039	$M>D$	0.008
Age of illness onset (years; Mean ± SD)	N.A.	27.51 ± 11.86	25.04 ± 9.74	25.09 ± 7.19	0.594	2	0.554		
Total illness year (Mean ± SD)	N.A.	8.38 ± 4.97	10.97 ± 10.42	14.34 ± 7.53	3.856	2	0.025	$D>E$	0.006
Duration of last episode (weeks; Mean ± SD)	N.A.	75.23 ± 73.73	5.12 ± 4.34	28.29 ± 38.58	14.464	2	0.0004	$D>M$	0.0002
								$E>D$	0.0002
								$E>M$	0.0001
Total number of manic/depressive episodes (Mean ± SD)	N.A.	5.53 ± 5.58	6.75 ± 5.31	10.42 ± 5.35	5.739	2	0.005	$D>M$	0.0002
								$D>E$	0.0002
								$M>E$	0.0001
CGI score (Mean ± SD)	N.A.	1.16 ± 0.37	5.21 ± 1.01	4.50 ± 0.75	292.345	2	0.0001	$M>E$	0.0003
								$D>E$	0.0002
								M > D	0.005
GAF score (Mean ± SD)	N.A.	88.81 ± 6.27	38.45 ± 9.39	45.00 ± 9.39	293.345	2	0.0003	$E>M$	0.0004
								$E>D$	0.0003
								$D=M$	0.079
HDRS score (Mean ± SD)	N.A.	1.91 ± 1.94	0.83 ± 1.62	24.00 ± 5.75	326.539	2	0.0003	$D>E$	0.0003
								$D>M$	0.0009
YMRS score (Mean ± SD)	N.A.	0.79 ± 1.69	30.37 ± 11.09	3.00 ± 0	124.557	2	0.0005	$M>E$	0.0001
Presence of psychosis/life-time (%)	N.A.	34.7	51.0	14.3	20.888[a]	2	0.0002	$M>D$	0.0005
Drug-free (%)	N.A.	8.3	5.6	7.4	8.512[a]	4	0.075		
Lithium use (%)	N.A.	57.6	63.6	20.0	3.931[a]	2	0.140	$E>M$	0.017
Duration of Li use (week) (Mean ± SD)		180.77 ± 143.99	66.31 ± 123.18	76.00 ± 83.76	3.160	2	0.056		
Li levels (mEq/L) (Mean ± SD)		0.88 ± 0.18	0.68 ± 0.27	0.57 ± 0.33	5.198	2	0.010		
Li dose (mg/day) (Mean ± SD)		1279.41 ± 403.91	1316.67 ± 443.0	1295.46 ± 568.09	0.772	2	0.470		
Valproate use (%)	N.A.	52.9	48.5	65.4	1.755[a]	2	0.416		
Duration of VA use (week) (Mean ± SD)		173.40 ± 102.99	80.68 ± 119.92	142.23 ± 136.32	1.353	2	0.276		
VA levels (ng/mL) (Mean ± SD)		62.79 ± 22.01	60.80 ± 29.79	64.64 ± 24.97	0.071	2	0.932		
VA dose (mg/day) (Mean ± SD)		1150.00 ± 257.25	1092.86 ± 417.80	985.71 ± 333.80	0.019	2	0.891		
AA use (%)	N.A.	45.9	75.0	61.9	7.057[a]	2	0.070		
Duration of AA use(week) (Mean ± SD)		71.56 ± 73.01	12.67 ± 19.73	65.13 ± 80.51	1.412	2	0.261		

[a] χ^2, H=healthy volunteers, E=euthymia, M=mania, D=depression, Li=lithium, VA=valproate, AA=atypical antipsychotic, NA=Not applicable, HDRS=Hamilton Depression Rating Scale, YMRS=Young Mania Rating Scale.

3. Results

3.1. Clinical features

The mean age was not different between patients and healthy controls. Females dominated the group with manic episode (65.7%). Age of illness onset did not differ among patients in different states of illness. Duration of current euthymia was longer than the duration of current depressive and manic episodes. CGI score was higher in mania than depression. The rate of lithium, valproate or atypical antipsychotic drug use, their doses and lenghts of use were comparable across the episodes. Serum lithium levels were higher in euthymic patients than those in mania. All demographic and clinical characteristics and related statistical values of the patients and healthy volunteers were given in Table 1.

3.2. Serum BDNF and GDNF levels in patients and healthy volunteers

The mean serum BDNF levels differed significantly among the groups ($F[3:155]=11.692$, $p=0.0006$). Serum BDNF levels were significantly low in both depressive (3639.58 ± 1395.09 pg/mL) and manic (4129.18 ± 1703.98 pg/mL) patients than patients in euthymia (6851.00 ± 3275.91 pg/mL) ($p=0.0003$ and $p=0.0001$ respectively) and healthy volunteers (5646.23 ± 2586.99 pg/mL) ($p=0.0006$ and $p=0.007$ respectively). Serum BDNF levels did not differ between healthy volunteers and eutyhmic patients ($p=0.310$), and between manic and depressive episodes ($p=0.782$) (Fig. 1(a)). Variance analysis revealed no significant difference between normal controls (112.61 ± 19.74 pg/mL) and patients in manic (122.36 ± 33.01 pg/mL), depressive (111.39 ± 24.23 pg/mL) or euthymic (121.06 ± 33.49 pg/mL) states of bipolar disorder ($F[3;103]=1.035$, $p=0.380$) (Fig. 1(b)). Serum GDNF levels were higher in early onset (19 years <) (134.23 ± 35.21) than late onset (19 years >) (116.44 ± 26.43) bipolar patients ($t[64]=2.218$, $p=0.030$) while serum BDNF levels were similar in both groups ($t[82]=-1.313$, $p=0.193$). There was no significant gender difference with regard to serum BDNF and GDNF levels in either patient or healthy groups. Neither the patients differed based on the presence or absence of life-time psychotic features.

3.3. GDNF/BDNF ratios in patients and healthy volunteers

GDNF/BDNF ratios differed significantly among the four groups ($F[3;96]=6.337$, df=3 $p=0.001$). Euthymic patients and healthy controls showed similar GDNF/BDNF ratios (0.021 ± 0.01 and 0.024 ± 0.01 respectively) ($p=0.812$). GDNF/BDNF ratios were significantly higher in manic episode (0.034 ± 0.01) compared to both euthymic state and healthy controls ($p=0.001$ and $p=0.007$ respectively). The difference between depressive episode (0.035 ± 0.02) and euthymia did not reach statistical significance although the ratio was higher in depression. ($p=0.082$). GDNF/BDNF ratio was comparable between depressive episode and healthy controls ($p=0.285$) and between the two illness states ($p=1.000$) (Fig. 1c).

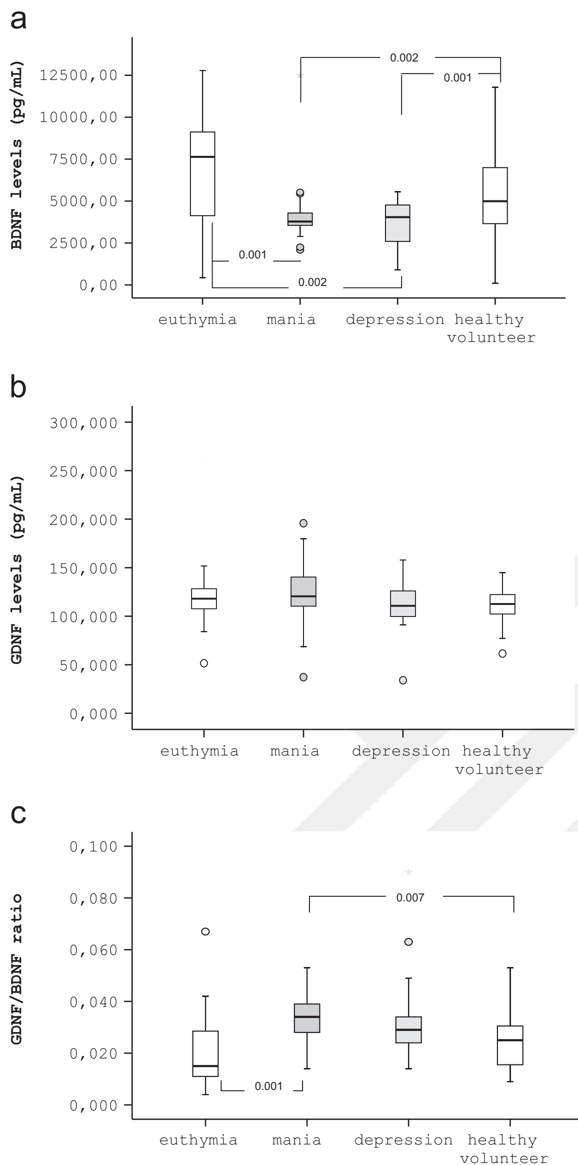

Fig. 1. (a)–(c): BDNF and GDNF serum levels and GDNF/BDNF ratio in patients and controls.

3.4. Correlations between clinical variables and BDNF and GDNF levels, and GDNF/BDNF ratio

3.4.1. In the entire group

Neither BDNF nor GDNF levels correlated with age in either patients or healthy individuals.There was no significant correlation between serum BDNF levels and age of illness onset, total number of manic or depressive episodes, and total illness years. However GDNF levels negatively correlated with age of illness onset ($n=63$, $r=-0.288$, $p=0.022$). BDNF levels showed negative correlations with HDRS ($n=95$, $r=-0.325$, $p=0.001$), YMRS ($n=74$, $r=-0.398$, $p=0.0001$) and CGI scores ($n=94$, $r=-0.306$, $p=0.003$), and a positive correlation with GAF scores ($n=67$, $r=0.415$, $p=0.0001$). GDNF levels also correlated negatively with HDRS scores ($n=95$, $r=-0.286$, $p=0.005$), but not correlated with YMRS, CGI, GAF scores, and total number of manic or depressive episodes and total illness years.GDNF/BDNF ratio had a positive correlation with YMRS ($n=55$, $r=0.381$, $p=0.004$) and CGI scores ($n=73$, $r=0.271$, $p=0.021$), and negative correlation with GAF scores ($n=66$, $r=-0.418$, $p=0.0001$). GDNF/BDNF ratio did not correlate with HDRS scores ($p=0.237$).

3.4.2. In euthymia

BDNF and GDNF levels were negatively correlated ($n=29$, $r=-0.491$, $p=0.007$) (Fig. 2(a)). Duration of euthymia (75.23 ± 73.73 weeks) was not correlated with either BDNF or GDNF levels ($p=0.718$ and $p=0.846$ respectively).

3.4.3. In manic episode

There was no correlation between BDNF and GDNF levels ($p=0.460$) (Fig. 2b). Duration of current manic episode (4.75 ± 4.14 weeks) did not correlated with either of the two trophic factors.

3.4.4. In depressive episode

There was no correlation between BDNF and GDNF levels ($p=0.543$) (Fig. 2c). Neither BDNF nor GDNF levels correlated with duration of current depressive episode.

3.5. Findings related to drug treatment and BDNF and GDNF levels

3.5.1. Comparison between drug-free, lithium treated or non-lithium treated patients

The frequencies of lithium, valproate or atypical antipsychotic use were comparable between different states of illness (Table 1). There was no significant difference in BDNF and GDNF levels between drug-free ($n=8$, 4553.12 ± 2238.65 pg/mL and $n=5$, 115.06 ± 12.88 pg/mL respectively), lithium treated ($n=59$, 5282.78 ± 2630.80 pg/mL and $n=50$, 119.63 ± 24.49 pg/mL respectively) or non-lithium treated ($n=30$, 4997.00 ± 3199.81 pg/mL and $n=24$, 116.05 ± 44.72 pg/mL respectively) patients and healthy controls ($p=0.583$ and $p=0.772$ respectively).

3.5.2. Correlations between BDNF or GDNF levels and doses, durations of treatment and serum levels of lithium and valproate

3.5.2.1. In euthymia. Serum lithium levels (0.88 ± 0.18 mEq/L) correlated positively with BDNF ($n=15$, $\rho=0.667$, $p=0.007$), negatively with GDNF levels ($n=13$, $\rho=-0.833$, $p=0.004$). A similar pattern with the serum valproate levels (62.79 ± 22.01) was observed, but not at statistically significant levels ($p=0.316$ and $p=0.416$ respectively) (Fig. 3a, b). Neither doses nor durations of lithium and valproate treatment correlated with BDNF and GDNF levels. Regression analysis confirmed that lithium levels predicted only GDNF levels negatively in euthymia ($R^2=0.691$, df=1, df2=11, $F=24.572$, B1 = −139.835, $p=0.0004$) but not BDNF levels at significant level ($p=0.084$) (Fig. 2a).

3.5.2.2. In manic and depressive episodes. Neither lithium and valproate doses nor their durations of treatment, and also valproate levels correlated with BDNF levels in manic episode. Lithium dose (985.71 ± 333.81 mg) correlated negatively with BDNF levels in depression, ($n=7$, $\rho=-0.787$, $p=0.036$). Serum lithium levels (0.68 ± 0.27 mEq/L) correlated positively with GDNF levels ($n=17$, $\rho=-0.489$, $p=0.047$) in mania. Regression analysis confirmed that lithium levels predicted GDNF levels positively in manic episode ($R^2=0.239$, df=1, df2=15, $F=24.572$, B1=55.2888, $p=0.047$) (Fig. 2b).

4. Discussion

Our findings confirmed that decreased BDNF level is a state marker for both manic and depressive episodes (Cunha et al., 2006: Fernandes et al., 2011; Kapczinski et al., 2008; Lin, 2009; Machado-Vieira et al., 2007; Palomino et al., 2006; Tramontina et al., 2009), and also is an indicator for the severity of the illness status (Fernandes et al., 2011). In contrast to some studies we

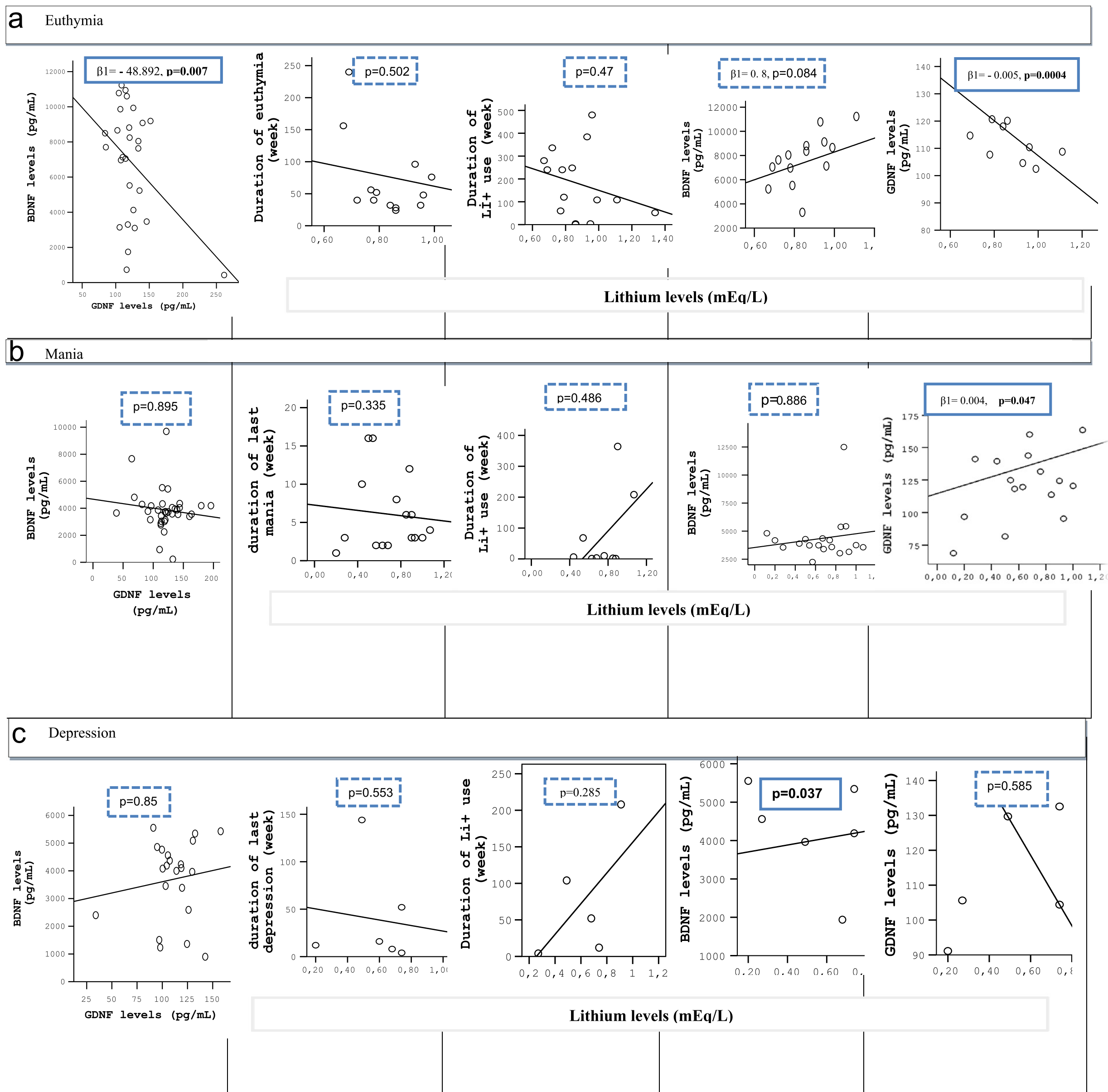

Fig. 2. (a)–(c): Regression curves of BDNF and GDNF levels according to the durations of the last episodes, duration of lithium use and lithium levels in euthymia, mania and depression.

could not detect any gender difference in relation to the BDNF and GDNF levels (Lommatzsch et al., 2005; Ozan et al., 2010). Neither was there a negative correlation between BDNF levels and age as shown previously (Erickson et al., 2010; Fernandes et al., 2011; Yatham et al., 2009). However, whether the alterations in expression of BDNF or any different primary pathology precede the development of the disease process in mood disorders is not clear (Tapia-Arancibia et al., 2004).

Despite higher mean of GDNF serum level in mania, we found no significant difference among the groups. This may be explained by type II error, and needed larger sample sized studies. The finding is inconsistent with previous studies reporting increased immunocontent ratio in manic and depressive episodes (Rosa et al., 2006), increased plasma GDNF levels in euthymia (Barbosa et al., 2011), decreased whole blood GDNF levels in mania (Takebayashi et al., 2005), decreased serum GDNF levels in mania and depression (Zhang et al., 2010), reduced blood cell expression of mRNA levels in depressive episode or in remission (Otsuki et al., 2008), and higher plasma GDNF concentration in late-onset depression (Wang et al., 2011). These discrepancies may be related to the variance in types of the study material such as plasma, serum or whole blood, and in sample size or age groups across different studies. Additionally, a transient increase of GDNF after injury has been reported in experimental studies (Saavedra et al., 2008; Vejsada et al., 1998). Such lack of consistency in GDNF related alterations may also be explained by the variance in the timing of blood sampling across the studies. The absence of any correlation between GDNF levels and age in our study is in line with some previously published studies (Barbosa et al., 2011; Diniz et al., 2012; Wang et al., 2011), but contradictory to some others (Tseng et al., 2013; Michel et al., 2008). The negative correlation with serum GDNF levels and age of illness onset in our study is consistent with a recent study (Tseng et al., 2013). Significantly higher GDNF levels in our early onset bipolar patients can be associated with an up-regulation of GDNF in early onset bipolar disorder where impaired synaptic strength due to glial dysfunction as seen in schizophrenia, may be expected (Moises et al., 2002). We observed significantly higher GDNF/BDNF ratio in

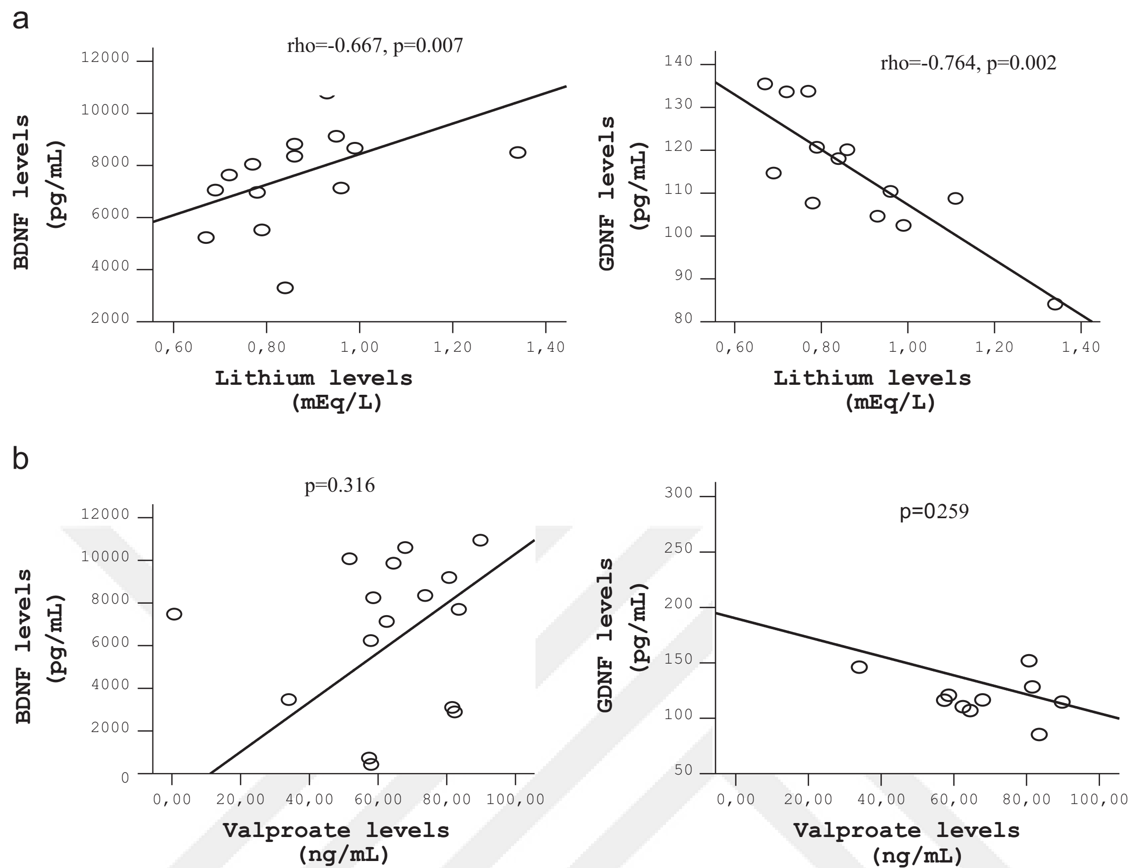

Fig. 3. (a) and (b) Correlations with lithium and valproate levels between BDNF and GDNF levels in euthymia.

mania compared to both healthy volunteers and euthymic patients. This result may be explained by either decreased levels of BDNF or increased levels of GDNF. If the increased ratio is BDNF dependant, a similar pattern could be expected in depression where they are evident. The absence of a similar significant ratio increase in the depressed group may be resulting from a small sample size. Neurons and glial cells were reported to have a bidirectional communication at the synapses to regulate synaptic plasticity (Allen and Barres, 2005; Bezzi and Volterra, 2001; Todd et al., 2006). Impaired glial cell functions in bipolar disorder (Rajkowska, 2000; Uranova et al., 2004) results in decreased synaptic glutamate uptake and extra cellular glutamate toxicity which has been consistently observed in mood disorders (Zarate et al., 2003), especially in bipolar patients in manic episode (Frye et al., 2007; Öngür et al., 2008; Yüksel and Öngur, 2010). Therefore, if the nonsignificant difference of GDNF levels among the groups is related to the type II error, and the higher GDNF/BDNF ratio in mania is GDNF dependant, our data showing statistically significant positive correlation between YMRS score and GDNF/BDNF ratio may reflect an increase in GDNF levels against possible glutamate toxicity in mania. Concomitant measurement of GDNF and glutamate levels in manic episode would clarify this assumption. Our result of negative association between GDNF levels and the severity of depressive episodes, in line with previous data (Pallavi et al., 2013) can be a further proof of our first assumption, namely, increase of GDNF may not be required in depressive episodes because of the reduced glutamate cycle in depression (Maddock and Buonocore, 2012; Yüksel and Öngür, 2010). The absence of significance difference in GDNF/BDNF ratio between depressive episode and both healthy controls and euthymic patients, in the presence of such difference in BDNF levels which is seen more prominently in bipolar depression than mania (Fernandes et al., 2009), may be explained by the existence of distinct pathogenesis between mania and depression. Nevertheless, since the BDNF levels are comparably decreased in mania and depression, and the GDNF/BDNF ratio in depressive episode had a trend to be higher from euthymia ($p=0.082$) which has been demonstrated to be significantly higher in mania, can be interpreted that depression and mania are a continuum rather than distinct phenomenon (Angst and Gamma, 2008; Mizuno et al., 2013; Phillips and Kupfer, 2013) at least in the context of trophic factors. Further studies are warranted to explain this hypothesis.

In our study, serum lithium levels showed a significantly positive correlation with BDNF levels, but a negative correlation with GDNF levels in euthymia. The first result supports the neurotrophic effect of lithium in accordance with previous data in experimental studies in rats (Angelucci et al., 2003; Frey et al., 2006; Fukumoto et al., 2001), in bipolar (de Sousa et al., 2011; Rybakowski and Suwalska, 2010; Tramontina et al., 2009) and depressive (Yoshimura et al., 2007) patients. However, the evidence associated with lithium's effect on GDNF levels is contradictory. Increased GDNF concentration by lithium was described in frontal and occipital cortices of the flinders resistant line rats (Angelucci et al., 2003). In contrast to this, acute or chronic lithium treatments were shown not to change GDNF protein expression in brain (Fukumoto et al., 2001), in spinal cord-drived progenitor cells (Su et al., 2009) and neural precursor cells (Qu et al., 2011) in rats. A negative correlation between lithium and GDNF levels in early Alzheimer patients was recently reported (Straten et al., 2011) similar to our observation in euthymic bipolar patients. In experimental studies, BDNF and GDNF were reported to act synergistically, but not simultaneously to rescue injured neurons (Vejsada et al., 1998; Zurn et al., 1996). Structurally dissimilar mood stabilizers, lithium and valproate were reported to act

similarly by regulating glutamate receptor expressions (Du et al., 2003), and providing a balance in tripartite synapse by probably controlling of glutamate toxicity (Cali and Bezzi 2012; Lee et al., 2007; Machado-Vieira et al., 2009; Perea and Araque, 2010). The present data does not provide enough evidence to assume that BDNF and GDNF act simultaneously due to lack of measurable effects that can be attributed to coinciding in time. Such measurements can be possible under controlled conditions where BDNF and GDNF can be manipulated as in animal studies. However our data suggests that BDNF and GDNF levels changed in opposite directions at least in euthymic patients using lithium, and the serum lithium levels predicted only GDNF levels but not the BDNF levels in the positive direction in mania, as confirmed by regression analysis. Further studies on drug-free patients are warranted to demonstrate whether the behavioral pattern between GDNF and BDNF as shown in our study is linked directly to the pathogenesis of bipolar disorder or to treatment effects.

5. Limitations

The first limitation of the current study is the small sample size in different episodes of bipolar disorder. However, evaluation of the study population by structured interviews, exclusion of the mixed and hypomanic episodes to verify accurate difference between manic and depressive states are the strengths of our study. The second limitation is that most of our patients were on drug tretment which is a potential source of bias. However, we showed comparable BDNF and GDNF levels between medicated and small sample sized drug-free patients which is in line with previous studies (Machado-Vieira et al., 2007; Tseng et al., 2013; Wang et al., 2011). Nevertheless, our findings of diverse effect of lithium on BDNF and GDNF levels to achieve euthymia, needs clarification in prospectively designed studies. Measuring the BDNF and GDNF levels in serum was our third limitation. Peripheral levels of BDNF was demonstrated to predict central levels (Hashimoto et al., 2004; Karege et al., 2002), but the correlation between central and peripheral levels of GDNF is not obvious (Kastin et al., 2003).

6. Conclusion

Our data suggest that lithium exerts its therapeutic action by an inverse effect on BDNF and GDNF levels, possibly by up-regulating BDNF and down-regulating GDNF to achieve euthymia, thus improving signaling pathways at tripartite synaptic level.

Role of funding source

Turkish Psychiatric Association.
Turkish Bipolar Disorder Association.
Turkish Lithium Association.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

We acknowledge to Hakan Aykan, MD for his valuable contributions to organize collection of blood samples.

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Psychiatry Research 236 (2016) 148–157

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Regulators of mitochondrial complex I activity: A review of literature and evaluation in postmortem prefrontal cortex from patients with bipolar disorder

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ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form
10 December 2015
Accepted 12 December 2015
Available online 17 December 2015

Keywords:
Bipolar disorder
Mitochondrial complex I
Mitochondrial regulators
Gene expression
Microarray
DJ-1

ABSTRACT

Phenomenologically, bipolar disorder (BD) is characterized by biphasic increases and decreases in energy. As this is a state-related phenomenon, identifying regulators responsible for this phasic dysregulation has the potential to uncover key elements in the pathophysiology of BD. Given the evidence suggesting mitochondrial complex I dysfunction in BD, we aimed to identify the main regulators of complex I in BD by reviewing the literature and using the published microarray data to examine their gene expression profiles. We also validated protein expression levels of the main complex I regulators by immunohistochemistry. Upon reviewing the literature, we found *PARK-7*, *STAT-3*, *SIRT-3* and *IMP-2* play an important role in regulating complex I activity. Published microarray studies however revealed no significant direction of regulation of *STAT-3*, *SIRT-3*, and *IMP-2*, but a trend towards downregulation of PARK-7 was observed in BD. Immunocontent of DJ-1 (*PARK-7*-encoded protein) were not elevated in post mortem prefrontal cortex from patients with BD. We also found a trend towards upregulation of DJ-1 expression with age. Our results suggest that DJ-1 is not significantly altered in BD subjects, however further studies are needed to examine DJ-1 expression levels in a cohort of older patients with BD.
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1. Overview of the literature

Bipolar disorder (BD) is a mood disorder associated with chronic course alternating between mania and depression, with symptoms characterized by alternating increases and decreases in energy and activity (Malhi et al., 2015). It is currently one of the main focuses of psychiatry research effort (Anderson et al., 2012). The World Health Organization (WHO) identifies BD as among the top ten leading cause of lifelong disability worldwide accounting for approximately 1–2% of the population affected (Woods, 2000). The underlying etiology of BD remains elusive, rendering translational strategies for therapeutic development very challenging (Andreazza and Young, 2014; Berk and Nierenberg, 2015).

Although the underlying pathophysiological mechanisms of BD remain largely unknown, as a phasic symptomatic disorder of energy, accumulating evidence is beginning to link BD to perturbations in mitochondrial functioning, especially complex I (Kato and Kato, 2000; Scola et al., 2013; Andreazza et al., 2010). For example, a review examining microarray findings revealed downregulation of genes encoding subunits of complex I such as *NDUFV1*, *NDUFS1*, *NDUFS8*, and *NDUFS7* in post-mortem frontal cortex and hippocampal samples from subjects with BD (Scola et al., 2013; Rajasekaran et al., 2015). These subunits that were found downregulated are located in the catalytic core of complex I

Abbreviations: BD, bipolar disorder; DHEA, Dehydropiandrosterone; DJ-1, protein deglycase; DOPAC 3,4, dihydroxyphenylacetic acid; FMN, flavine mononucleotide; Grx2, glutaredoxin 2; GSH, glutathione; 6-OHDA, 6-hydroxydopamine; IMP-2, insulin-like growth factor 2 [IGF2] mRNA-binding protein 2; NO, nitric oxide; PARK-7, Parkinson disease protein 7; ROS, reactive oxygen species; SCZ, schizophrenia; SIRT, NAD-dependent deacetylase sirtuin; STAT-3, signal transducer and activator of transcription 3

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http://dx.doi.org/10.1016/j.psychres.2015.12.015
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involved specifically in the transportation of electrons from NADH to ubiquinone (Scola et al., 2013; Hirst, 2013). Ubiquinone dysregulation is thought to influence mood disorders via pathways that include cytoprotection, hydrogen peroxide formation as well as gene, cellular metabolism and bioenergetic regulation (Morris et al., 2013). In contrast, patients with SCZ presented a non-specific alteration, which included increased and decreased gene expression levels distributed throughout complex I (Scola et al., 2013). This suggests that there is a disease-specific biochemical alteration in complex I activity that may confer a marked susceptibility to electron leakage in BD subgroup (Scola et al., 2013). Consistent with these microarray findings, NDUFS7 protein expression levels and complex I activity were found significantly reduced in the prefrontal cortex (PFC) area from patients with BD, but not in SCZ (Andreazza et al., 2010). Complex I dysfunction could translate into increased electron leakage directly to oxygen which would ultimately lead to the formation of reactive oxygen species (ROS) that may induce oxidative stress in BD (Halliwell, 1992; Clay et al., 2011; Brown et al., 2014), which in turn could be reflected by decreased mitochondrial complex I activity in BD (Moylan et al., 2014; Morris and Berk, 2015).

Despite several recent reports on various susceptibility genes, no study has yet identified genetic alterations in mitochondrial regulators that support decreased complex I activity in BD. At present, there is accumulating evidence pointing towards regulation of complex I by four players: protein deglycase (DJ-1), signal transducer and activator of transcription 3 (STAT-3), NAD-dependent deacetylase sirtuin (SIRT-3), and insulin-like growth factor 2 [IGF2] mRNA-binding protein 2 (IMP-2). There is less conclusive evidence that implicates other possible regulators, such as hormones, cytokines, monoamines such as dopamine, and metabolites (Alberio et al., 2014). There are two levels of complex I regulation: (1) positive regulation which directly or indirectly enhances complex I activity or (2) negative regulation which directly or indirectly decreases complex I activity (Fig. 1). Thus, direct or indirect regulation of complex I by these regulators and inhibitors may underlie its neuroprotective effects, drive phasic oscillations of mitochondrial activity that might underpin core phenomenology or may contribute to the development of BD.

1.1. Inhibitors of complex I activity

Dysregulation of the dopaminergic system is implicated in BD, where elevated levels of synaptic dopamine were postulated to drive mania-like characteristic of this disorder (Berk et al., 2007). Conversely, agents that drive increase in dopamine were used to treat depression (Dell'Osso et al., 2013). High levels of dopamine can induce toxicity in neurons due to its spontaneous oxidation that could result in the generation of toxic ROS and metabolites such as hydrogen peroxide and quinones (Hastings et al., 1996). Dopamine may be metabolized either enzymatically by monoamine oxidase A to form 3,4 dihydroxyphenylacetic acid (DOPAC) with hydrogen peroxide (Maker et al., 1981), or non-enzymatically by hydrogen peroxide induced-hydroxylation in the presence of ferrous to form 6-hydroxydopamine (6-OHDA) (Graham, 1978). Dopamine may also undergo spontaneous oxidation to generate reactive quinone products owing to the fact that it is already under high oxidative stress due to its metabolism (Jana et al., 2011; Jana et al., 2007). A catechol moiety in dopamine exists that is pivotal for its direct interaction with complex I, suggesting that

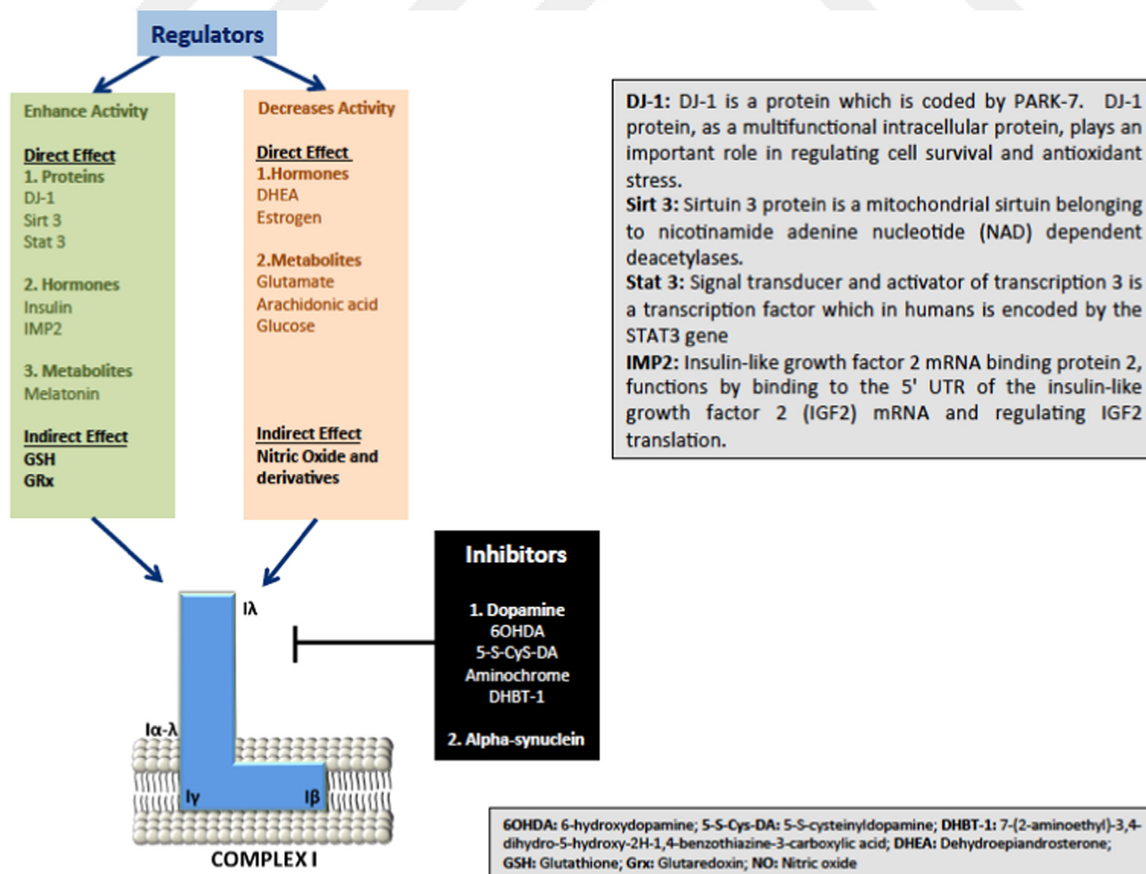

Fig. 1. Summary of regulators and inhibitors of complex I. Regulators that enhances activity with direct effect: DJ-1, Sirt 3, Stat 3, insulin, IMP-2; and indirect effect: GSH and GRx. Regulators that decreases activity with direct effect: DHEA, estrogen, glutamate, arachidonic acid, glucose; and indirect effect: nitric oxide and derivatives. Inhibitors that blocks activity: 6OHDA, S-S-Cys-DA, aminochrome, DHBT-1, and α-synuclein.

mitochondrial complex I is a potential target for dopamine (Ben-Shachar et al., 2004). Reactive quinones generated from 6-OHDA rather than dopamine itself appear to be involved in long-term direct inhibitory effect of complex I activity (Khan et al., 2005; Glinka and Youdim, 1995; Iglesias-Gonzalez et al., 2012), while DOPAC produces marginal complex I inhibition (Jana et al., 2007; Khan et al., 2005; Narendra et al., 2010). However, desferrioxamine and melatonin was found to prevent 6-OHDA-induced inhibition of complex I activity by inducing a conformational change that is unfavorable for dopamine binding (Ben-Shachar et al., 1991; Glinka et al., 1996; Glinka et al., 1998; Dabbeni-Sala et al., 2001). Collectively, these results support the role of dopamine in mitochondrial complex I dysfunction and could have important implications in BD.

Furthermore, other non-dopamine related compound such as α-synuclein is also known to inhibit complex I activity (Loeb et al., 2010). α-synuclein plays a key role in maintaining synaptic vesicles in presynaptic terminals and regulating the release of dopamine (Sidhu et al., 2004). One effect of lithium is to prevent the effects of elevated α-synuclein on oxidatively driven cell death (Kim et al., 2011). The inhibitory effect of α-synuclein on complex I seems to be dose-dependent and affects certain regions of the brain, suggesting that α-synuclein may be a potential factor underlying decreased neuronal densities observed in BD (Liu et al., 2009, Savitz et al., 2014, Gigante et al., 2011).

1.2. Negative regulators of complex I activity

In addition to the inhibitory effects of dopamine and α-synuclein, other molecules such as hormones, metabolites and nitric oxide derivatives can negatively regulate complex I activity, directly or indirectly to decrease its activity.

Hormonal imbalances significantly contribute to mood fluctuations in BD (Frey and Dias, 2014). For example, a recent review examining the role of estrogen in BD reported low serum estrogen levels in women with postpartum psychosis, and that estrogen treatment resulted in a significant improvement of these symptoms (Meinhard et al., 2014). However this hypothesis was questioned because another study reported that estradiol and tamoxifen were able to directly target the flavine mononucleotide site of complex I, leading to increased ROS production and decreased mitochondrial respiratory activity (Moreira et al., 2006). There is a lack of agreement in the literature evaluating the possible role of these hormones in triggering or maintaining the psychotic symptoms in BD through complex I interaction, although clinical trial data of estrogenic agents in schizophrenia and mania suggests a beneficial effect (Kulkarni et al., 2014; Kulkarni et al., 2015; Kulkarni et al., 2010). Furthermore, alterations in arachidonic acid signaling are implicated in BD because administration of lithium, antipsychotics and anticonvulsants were able to decrease arachidonic acid turnover in the brain phospholipids of rats (Rapoport 2014; Rapoport and Bosetti, 2002; Chang et al., 2001). Although the mechanisms by which this occurs is unclear, it was shown that arachidonic acid directly decreased complex I and complex III activity and promoted ROS production, suggesting its potential role in inhibiting mitochondrial respiratory function (Cocco et al., 1999; Takeuchi et al., 1991). Other metabolites such as glucose and glutamate signals were also effective at directly downregulating complex I function; however their implications in BD are unknown (Sugiyama et al., 2007; Cannino et al., 2012).

While findings for direct negative regulators are not well established, much attention has been diverted towards indirect negative regulators of complex I, such as nitric oxide (NO) and its derivatives. There is emerging evidence that mitochondrial oxidative phosphorylation generates reactive oxygen and nitrogen species as undesirable byproducts that may accumulate and trigger oxidative damage (Murray et al., 2003). The important reactive nitrogen species (RNS) in the mitochondria are NO and peroxynitrite (Murray et al., 2003). Superoxide radicals, produced by electron leakage directly to oxygen, may react rapidly with NO to produce highly reactive peroxynitrite (Poderoso et al., 1999). Peroxynitrite is a very strong oxidant that can readily permeate through mitochondrial membrane and cause nitrosative damage to important mitochondrial components via interaction with thiol and hydroxyl groups of cysteine, phenylalanine, and tyrosine of mitochondrial proteins (Marla et al., 1997; Beal, 2002; Beckman and Koppenol, 1996; Ramachandran et al., 2004). Mitochondrial oxidative phosphorylation appears to be modestly inhibited via this interaction. For example, tyrosine containing thiol groups of complex I can interact with peroxynitrite to generate S-nitrosothiol derivatives, resulting in irreversible inhibition of the complex that cannot be recovered in presence of thiol reducing agent, dithiothreitol (DTT) (Murray et al., 2003; Poderoso et al., 1999; Ramachandran et al., 2004; Riobo et al., 2001; Brown and Borutaite, 2004). Covalent modification of tyrosine residues on NDUFS2, NDUFS8, NDUFB4, and NDUFA6 of complex I were found most notable (Murray et al., 2003). Nitrosation induced-complex I inhibition can also cause subsequent increase in ROS production (Borutaite and Brown, 2006). This could explain some of the reported events observed in BD such as neuroinflammation, dysfunction of dopaminergic neurons, mitochondrial dysfunction and consequently decreased energy production, and neuronal cell death. These may also be mediated through the persistent exposure of macrophages to excessive levels of NO or by the selective inhibition of complex I subunits through irreversible S-nitrosylation and nitration (Chinta and Andersen, 2011; Clementi et al., 1998; Yamamoto et al., 2002; Choi et al., 2009; Davis et al., 2010). DOPAC and NO induced-complex I inhibition were also found to result in early depletion of glutathione (GSH) levels in dopaminergic neurons (Nunes et al., 2011; Hsu et al., 2005). However, high expression of superoxide dismutase, another antioxidant defense element, were able to significantly reduce complex I nitration and subsequently reestablish mitochondrial homeostasis and bioenergetics (Davis et al., 2010).

1.3. Positive regulators of complex I activity

While negative regulators decreases complex I activity, other proteins, hormones, and metabolites enhance the activity of complex I directly or indirectly, and are referred to as positive regulators. For instance, glutathione (GSH), which makes up the main intrinsic component of the antioxidant system (Mari et al., 2009), was found significantly impaired in individuals with BD (Rosa et al., 2014; Morris et al., 2014). GSH plays an important role in the brain by detoxifying reactive quinones-derived from dopamine oxidation involved in complex I inhibition, thereby decreasing inhibition and indirectly enhancing complex I activity (Jana et al., 2011; Mari et al., 2009). GSH maintains the integrity of complex I and indirectly enhances its activity by either protecting the complex against irreversible thiol s-nitrosylation to prevent further oxidation of critical thiols or by eradicating NO from nitrosylated thiols that are already present on complex I (Chinta and Andersen, 2011; Clementi et al., 1998; Martin and Teismann, 2009). Decreased GSH expression in the adult dopaminergic midbrain neurons was found to trigger mitochondrial damage and nigrostriatal cell loss via GSH depletion-induced complex I inhibition (Jain et al., 1991; Chinta et al., 2007). Significant depletion of total GSH expression within dopaminergic PC12 cells was found to selectively inhibit complex I through oxidation of tyrosine containing thiols by NO species (Hsu et al., 2005; Jha et al., 2000). Similarly, peroxynitrite was able to induce significant complex I inhibition in N27 cells following GSH depletion (Chinta and

Andersen, 2006; Bharath and Andersen, 2005). Both cases were further supported by increased nitrosylation to mitochondrial proteins and the ability of DTT to reversibly remove these nitrosylated modifications on tyrosine residues of complex I (Jha et al., 2000; Chinta and Andersen, 2006; Bharath and Andersen, 2005). To alleviate reduced complex I activity, upregulation of γ-glutamyl transpeptidase (GGT) in an effort to increase cysteine availability for GSH production was shown in Parkinsonian substantia nigra (Chinta et al., 2006). In addition, oxidation of GSH to glutathione disulfide (GSSG) in the presence glutaredoxin 2 (Grx2) was found to effectively mediate complex I inhibition via glutathionylation (Taylor et al., 2003; Beer et al., 2004). Grx2 also possesses an antioxidant role by catalyzing deglutathionylation, which is important for reversing thiol modifications of complex I (Beer et al., 2004). An in vivo study demonstrated that downregulation of Grx2 led to partial loss of complex I activity, suggesting an important role of Grx2 in maintenance and enhancement of complex I activity (Beer et al., 2004; Karunakaran et al., 2007). Glutathione deficiency in dopaminergic neurons unsurprisingly led to Grx2 inhibition and subsequent complex I inhibition by decreasing the iron-sulfur assembly (Lee et al., 2009). Altogether, this suggests the neuroprotective role of GSH in preventing nitrosative damage in complex I (Hsu et al., 2005; Heales and Bolanos, 2002), and therefore GSH may be an important candidate regulator to enhance mitochondrial function in BD. Other positive regulators of complex I include: DJ-1, SIRT-3, STAT-3, insulin and IMP2, which are discussed in more detail below.

1.3.1. DJ-1

Like α-synuclein, DJ-1 also has direct effects on mitochondrial complex I activity (Sai et al., 2012). DJ-1, coded by the *PARK-7* gene was first characterized as an oncogenic protein (Nagakubo et al., 1997). Recently, it is known as a multifunctional protein that plays a significant role in regulation of transcriptional activity (Blackinton et al., 2009), has anti-apoptotic properties (Haigang et al., 2012), is a chaperone molecule folding proteins into a 3-dimensional shape and protects against oxidative stress (Taira et al., 2004) through regulation of mitochondrial homeostasis (Takahashi-Niki et al., 2012). DJ-1 is highly expressed in the astrocytes, which are specialized glial cells that defend surrounding neurons against oxidative stress induced-neuronal death by secreting antioxidants (Bandopadhyay et al., 2004; Ashley et al., 2009; Mullett and Hinkle, 2011). In cells, DJ-1 plays a neuroprotective role by translocating to the mitochondria and directly binding to NDUFA4 and ND1 subunits of mitochondrial complex I to maintain its activity and integrity (Takahashi-Niki et al., 2012; Hayashi et al., 2009). Loss of DJ-1 was found to directly inhibit complex I by blocking electron transport (Kwon et al., 2011). For example, DJ-1 knockdown in astrocytes was found significantly less neuroprotective compared to functional DJ-1 astrocytes because DJ-1 deficiency impaired the selective capacity of astrocytes to protect neurons against complex I inhibiting pesticides (Mullett and Hinkle, 2011). This is consistent with previous findings in which DJ-1 overexpression in astrocytes was able to rescue the effects of rotenone induced-complex I inhibition (Mullett and Hinkle, 2009). Moreover, other reports showed that mice deficient in DJ-1 were more susceptible to 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyrindine (MPTP)-induced toxicity and DJ-1 knockout cells showed reduced complex I activity and resistance to herbicide paraquat-mediated ROS production as a result of mitochondrial complex I dysfunction (Kwon et al., 2011; Kim et al., 2005). Interestingly, complex I activity was found to be partially restored following DJ-1 ectopic expression, and overexpression provided neuroprotection against rotenone induced-apoptosis (Kwon et al., 2011; Gao et al., 2012). Moreover, DJ-1 was able to prevent dopaminergic neuronal cell death induced by 6-OHDA (Inden et al., 2006). These findings suggest that DJ-1 directly enhances complex I activity and alterations in its expression levels may be implicated in mitochondrial dysfunction and oxidative damage in BD.

1.3.2. SIRT-3

SIRT-3 is the primary mitochondrial nicotinamide adenine nucleotide (NAD) dependent deacetylase (Weir et al., 2013). It plays an important role in regulating metabolism, stress responses, cell survival and signaling (Kong et al., 2010; Sack and Finkel, 2012). SIRT-3 directly regulates mitochondrial homeostasis including response to oxidative stress (Bause and Haigis, 2013). SIRT-3 deficiency in cells was found to prompt excessive levels of acetylated modifications to mitochondrial subunits of complex I, particularly NDUFA9 which in turn caused significant reduction in ATP production (Ahn et al., 2008). SIRT-3 reversibly binds to and regulates the deacetylation of NDUFA9, thereby enhancing complex I activity (Ahn et al., 2008). As expression of SIRT-3 regulates the acetylation levels and activity of complex I, it may be implicated in BD (Ahn et al., 2008).

1.3.3. STAT3

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor encoded by the *STAT3* gene (Gough et al., 2009). STAT3 is activated by cytokines, growth factors, interferons, IL-5, IL-6 and leptin (Levy and Lee, 2002). In addition to its involvement in regulating gene transcription, cell growth and apoptosis, STAT3 was recently identified as an important regulator of mitochondrial respiratory activity since it localizes to the mitochondria and interacts with complex I and II (Wegrzyn et al., 2009). Selective impairment of complex I and complex II activities were observed in STAT3 deficient cells, suggesting that STAT3 plays an important role in the normal functioning of the ETC (Wegrzyn et al., 2009). The exact mechanism of STAT3 interaction with complex I and complex II is not clearly known. However it was shown that the cellular ratio of complex I/II to mitochondrial STAT3 is 10^5, suggesting that STAT3 is modulating oxidative phosphorylation through transcriptional regulation rather than direct protein interaction between complex I/II and STAT3 (Phillips et al., 2010). More recently, decreased levels of complex I, and severely damaged mitochondria were observed in a HL-1 STAT3 knock down cell line (Elschami et al., 2013). Collectively, these results suggest that decreased complex I activity due to STAT3 deficiency is a possible explanation for damage to mitochondrial proteins, and these consequences may be implicated in BD.

1.3.4. Insulin and IMP2

Insulin is a hormone that can directly act on complex I to enhance its activity. Individuals with BD have an increased likelihood of being diagnosed with type II diabetes (Calkin et al., 2013). Hyperglycemia can induce a significant reduction in complex I activity (Remor et al., 2011). However, insulin was able to directly improve complex I activity, suggesting the importance of insulin on the maintenance of mitochondrial homeostasis under diabetic conditions (Remor et al., 2011). Insulin resistance is also associated with mitochondrial dysfunction (Petersen et al., 2003). In addition, another gene, insulin-like growth factor 2 mRNA binding protein 2 (IMP-2) was shown to play an important role in regulating mitochondrial oxidative phosphorylation (OXPHOS) (Janiszewska et al., 2012). IMP2 is involved in the nuclear and mitochondrial control of disaccharide utilization, upregulates insulin-like growth factor 2 (Mu et al., 2015), and plays a role in neuronal differentiation (Fujii et al., 2013). IMP2 also regulates OXPHOS by binding to mRNA that encodes for complex I and complex IV subunits. IMP2 deficiency in gliomaspheres was able to induce a significant reduction and impairment in complex I activity, suggesting its role in enhancing complex I function (Janiszewska et al., 2012).

The objective of this study was thus to identify these main regulators of mitochondrial complex I in BD using publicly available microarray data, followed by confirmation of protein expression levels using immunohistochemistry. We hypothesized that, when compared to non-psychiatric controls, subjects with BD would have decreased expression of mitochondrial complex I regulators, DJ-1, STAT3, SIRT3, and IMP-2.

2. Methods

2.1. Post-mortem brain samples of microarray data

We used published microarray data from two post-mortem brain collections of the Stanley Medical Research Institute (SMRI) Online Genomic database where independent studies were conducted (https://www.stanleygenomics.org).

The first collection was from the Stanley Neuropathology Consortium, which includes brain tissue samples from various regions of the brain. The consortium collection contained brain samples from 60 subjects divided into four cases, 15 individuals each with BD, schizophrenia, major depressive disorder without psychotic features, and normal controls. These groups were matched for age, sex, race, post mortem interval (PMI), mRNA yield, pH, and side of the brain. Exclusion criteria included subjects over age 65 and poor mRNA yield. Further details of this collection have been published elsewhere (Torrey et al., 2000). Brain regions from this collection included the prefrontal cortex (Brodman Area 46, 6 8/9, 10) and the cerebellum, which were provided to a number of researchers to perform RNA extraction and microarrays.

The second collection was from the Stanley Array collection, which included several areas of the brain for each subject. The array collection contained brain samples from 105 subjects divided into three well-matched groups, 35 individuals each with BD, schizophrenia, and unaffected controls (http://www.stanleyresearch.org/brain-research/array-collection). Subjects excluded were similar to those for the Neuropathology Consortium. Regarding microarrays, samples from Brodmann area 46 of the prefrontal cortex were used from the array collection. Independent investigators performed RNA processing and microarrays analysis.

2.2. Cross-study analysis of microarray studies regarding mitochondrial complex I regulators

The cross study analysis included 9 Affymetrix, 1 Codelink, 1 Agilent, and 1 custom cDNA microarray studies. Twelve independent investigators performed and provided their microarray data to the SMRI. Twelve studies included on the SMRI online genomics database (http://www.stanleygenomics.org) were selected based on the number of patients investigated, the type of platform used, and the quality of the data. Investigators involved in microarray studies were assigned a specific study ID–1: Altar A, 2: Altar C, 3: Bahn, 4: Chen, 5: Dobrin, 6: Feinberg, 7: Kato, 9: Altar B, 10: Sklar B, 12: Vawter, 13: Young, 14: Sklar A. Additional information about sample can be found at SMRI Online Genomics Database and Choi et al. (2008).

Forest plots for each gene (*PARK-7, STAT3, SIRT-3, and IMP-2*) were obtained from the SMRI online genomics database to identify the significance of each gene based on a consensus fold change. The consensus fold change for each gene reflects a weighted combination of all individual studies' fold changes and standard error for the microarray probes that plot each gene across the studies. The weighted mean of individual fold changes is equal to $1/SE_i$, where SE_i is the standard error of the i'th probe for that gene. Therefore, each gene has a weighted mean of fold change and 95% confidence interval. More information regarding cross-study analysis of disease can be found at the SMRI online genomics database (https://www.stanleygenomics.org). Significant differentially expressed genes were identified as those with a fold change (FC) < -1.1 indicative of downregulation or FC > 1.1 indicative of upregulation, and a non-stringent *p*-value of $p < 0.05$ was used. Combined, array, and consortium analysis for BD with overall fold changes and *p*-values were obtained for each gene.

Table 1
Demographic and clinical characteristics of postmortem brain samples.

Characteristic	Group [mean (SD)]		
	Schizophrenia	Bipolar disorder	Control
Age	76.8(11.5)	77.3(10.9)	79(9.2)
PMI	19.4(10.4)	21.3(8.2)	21.3(5.7)
Sex, female:male	6:4	6:4	5:4

Table 2
Mean DJ-1 densities in prefrontal cortex from patients with BD or SCZ or non-psychiatric controls.

	Schizophrenia	Bipolar disorder	Control
M	0.39	0.36	0.38
SD	0.061	0.082	0.057

2.3. Post-mortem prefrontal cortex samples

Frozen postmortem PFC samples were obtained from The Harvard Brain Tissue Resource Center [Brodmann areas (BA10)]. Samples consisted of subjects from bipolar disorder ($n = 10$), schizophrenia ($n = 10$) and non-psychiatric controls ($n = 9$). The sections were closely matched for age, sex and post mortem interval (PMI). Additional demographic parameters and PMI for each group are provided in Table 2. Two senior psychiatrists performed psychiatric diagnosis retrospectively by applying the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria. Sections with good structural integrity were used for our experiments. All samples were numerically coded, stochastically arranged, and kept consistent throughout the experiment. Experimenters were kept blind to group identity until all experiments and quantifications were completed.

2.4. Analysis of immunocontent of DJ-1 in postmortem prefrontal cortex from patients with bipolar disorder

DJ-1 immunoreactivity in the brain sections was examined by measuring fluorescence density. Sections were fixed with 4% paraformaldehyde for 15 minutes prior to any labeling. Sections were then washed 3 times with PBS and were blocked for 1 hour in PBS containing 0.3% Triton-X-100 (PBS-T) and 10% goat serum. DJ-1 primary antibody (1:100) was added to the sections and these were incubated overnight for 48 h in PBS-T at 4 °C. On the following day, sections were washed 3 times in PBS-T and incubated with secondary antibody (1:200, Alexa Fluor™ 568 Goat Anti-Rabbit IgG) for 2 hours at room temperature. Labeled sections were mounted in Fluoromount and were covered for microscopy.

2.5. Confocal microscopy and image analyses

Fluorescently labeled sections were examined at 20x magnification using a confocal laser-scanning microscope. For each section, images of 5 fields were captured, as the intensity and threshold settings were kept consistent. The fluorescence densities

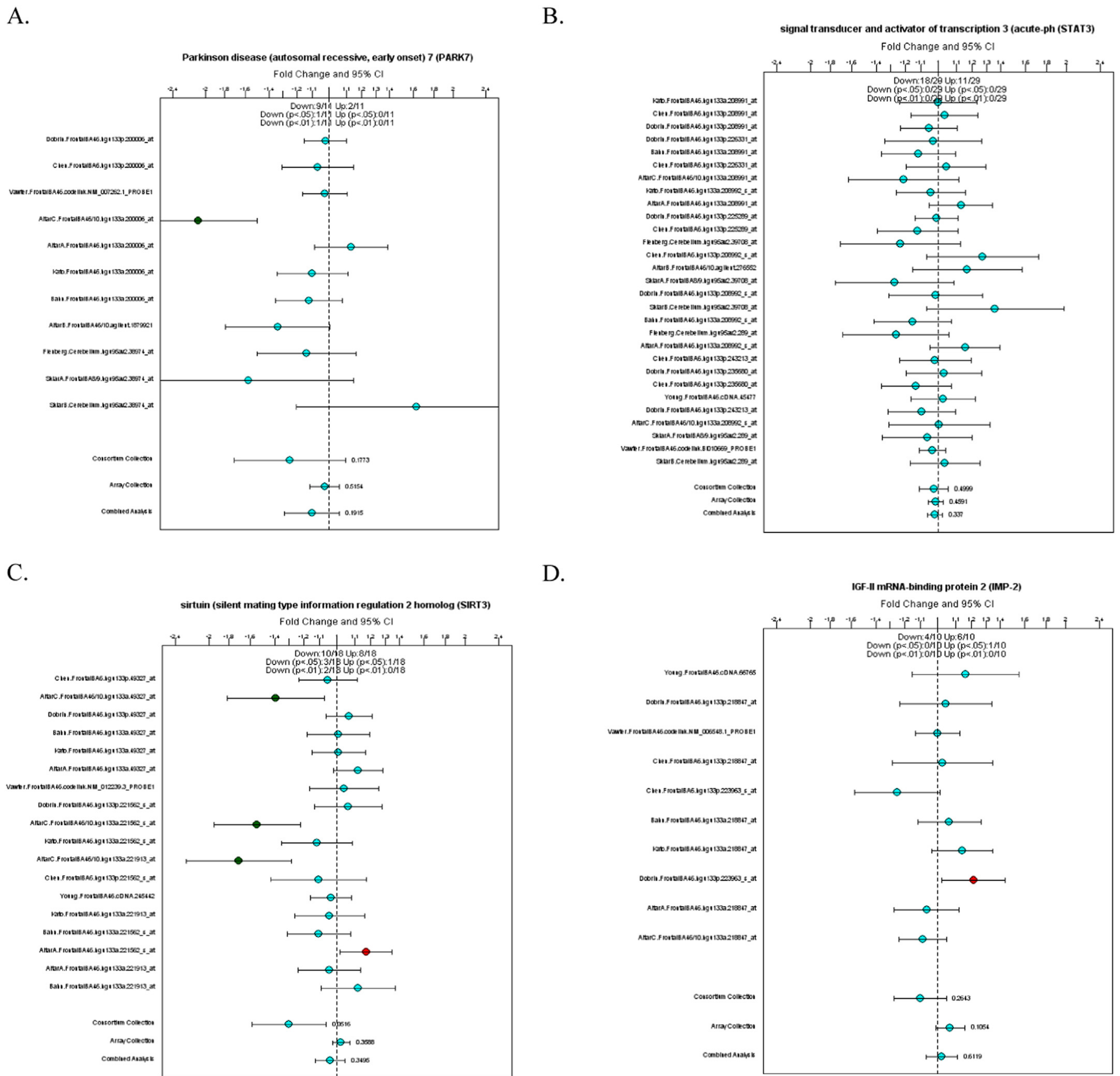

Fig. 2. Microarray data of candidate complex I regulators. A. PARK-7, B. STAT3, C. SIRT3, D. IMP-2. Plots with fold changes and 95% confidence intervals are depicted on the horizontal axis. Consortium collection, array collection, and combined analysis are shown on the bottom of each plot with weighted fold change and 95% confidence intervals.

of each DJ-1 positively labeled cell were measured and quantified manually using the Image Pro Plus software.

3. Results

3.1. Cross-study analysis revealed trend towards downregulation of PARK-7 in BD

We combined gene expression profiles of candidate complex I regulators that may be associated with decreased complex I activity in BD. Particularly, we evaluated the expression profile of complex I regulators (*PARK-7, STAT3, SIRT-3, IMP-2*) in BD using large fold change magnitudes and a non-stringent *p*-value ($p < 0.05$). Among the set of genes, we identified one gene that was almost consistently downregulated across multiple independent studies (Fig. 2A). When the analysis was segregated based on individual studies, the gene *PARK-7* was significantly downregulated ($p < 0.01$) in AltarC study with greater than two FC. Furthermore, across the 11 studies, the trend of expression was decreased for 9 of the 11 studies. The combined analysis reported a FC of -1.102 at $p=0.1915$, suggesting a non-significant downregulation of the *PARK-7* gene by a factor of greater than 1 in subjects with BD. Analysis from the consortium collection revealed a higher magnitude fold change of -1.25 at $p=0.1773$ compared to the combined analysis. The combined FC of -1.102 was also within or overlapped with the confidence intervals across all individual study results, which also indicates a non-significant but

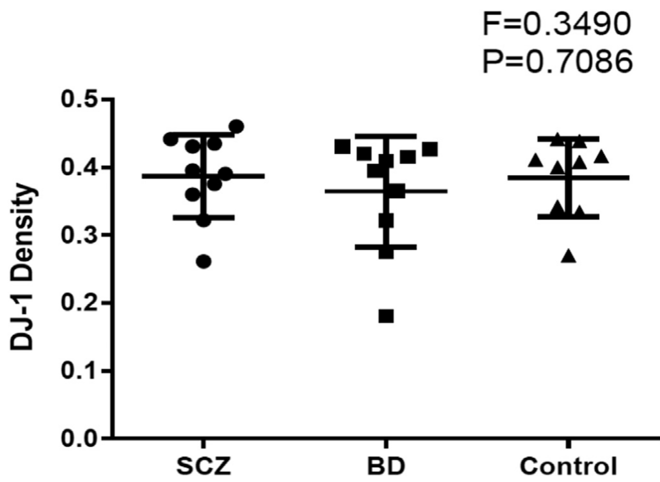

Fig. 3. Levels of DJ-1 expression in post-mortem prefrontal cortex from patients with SCZ, BD and non-psychiatric controls.

consistent trend towards downregulation of *PARK-7* in BD.

Interestingly, *SIRT3* was significantly downregulated at $p < 0.05$ in three of the studies by AltarC and upregulated at $p < 0.05$ in one study by AltarA. On the other hand, *IMP-2* was significantly upregulated at $p < 0.05$ in one study by Dobrin. But overall, the genes *STAT3, SIRT-3, and IMP-2* were not significantly differentially regulated since the combined analysis showed that the weighted fold changes were all close to zero and the p-values were greater than that of *PARK-7* ($p = 0.337$ for *STAT3*; $p = 0.3495$ for *SIRT3*; and $p = 0.6119$ for *IMP-2*) (Figs. 2B, C, D). Furthermore, individual studies for these genes were also regularly distributed about the vertical line, indicating inconsistencies across the independent studies. For example, the direction of regulation was down for 18 of the 29 studies for *STAT3*, down for 10 of the 18 studies for *SIRT-3*, and up for 6 of the 10 studies for *IMP-2*.

3.2. Immunocontent of DJ-1 is not elevated in post mortem prefrontal cortex from patients with BD

One-way analysis of variance revealed no statistical significant difference in mean DJ-1 expression levels between groups, $F(2, 27) = 0.35$, $p = 0.71$ (Table 1, Fig. 3). There were also no significant correlation found between PMI and DJ-1 density, $r = -0.00031$, $n = 29$, $p = 0.999$, two tails (Fig. 4A); and age and DJ-1 density (Fig. 4B), $r = +0.361$, $n = 29$, $p = 0.055$, two tails. Furthermore, no significant difference in mean DJ-1 densities were observed between males and females $t(27) = 1.95$, $p = 0.099$ (Fig. 4C).

4. Discussion

BD is a very complex illness with genetics being a significant contributing factor. However, no single gene has been consistently identified, making it challenging to come into a general consensus. Exploring gene expression may help uncover genes that are up- or downregulated in BD and thereby provide molecular insight on its genetic foundation that can help explain molecular and regulatory mechanisms in a pathway involved in the pathophysiology of BD. Gene expression studies of the prefrontal cortex from BD subjects have been widely conducted owing to the fact that most meaningful findings were detected in this brain region. However, there has been a lack of consistent findings across microarray studies as a result of small effective sample size and clinical heterogeneity, both of which can underestimate the variances and inflate the risk of concluding a false-positive effect. Combining the microarray studies of BD might offer advantages because it allows for the detection of gene expression patterns with increased statistical power to uncover consistent fold changes and significant effects when multiple independent studies are combined and also allows for exploration of specific pathways of interest.

Many proteins, hormones, and metabolites are involved in the regulation of complex I activity. The extent to which complex I activity is increased or decreased depends partly on the levels of expression of these molecules. To date, no studies have identified genetic alterations in mitochondrial regulators that support decreased complex I activity in BD. However, upon reviewing the literature and evaluating the publicly available microarray studies, converging evidence implicates regulation of complex I by several genes including *PARK-7, STAT3, SIRT-3,* and *IMP-2.* Therefore, our goal was to utilize gene expression of these mitochondrial regulators to understand gene regulation of complex I activity. In the current cross-study analysis, *STAT3*, *SIRT-3* and *IMP-2* were found not significantly regulated. However, we tested whether DJ-1 expression was downregulated in BD subjects since a positive, but insignificant trend towards downregulation of *PARK-7* was observed in the microarray studies. Here, we report no significant difference in mean DJ-1 densities between the groups, which confirm the findings of the microarray analyses, and suggests that DJ-1 is not significantly differentially regulated in BD subjects.

Although our immunohistochemical data revealed no significant difference in DJ-1 immunocontent between the group means, our correlation data that assessed the relationship between DJ-1 expression and age revealed a *p*-value just slightly above the significance level ($r = 0.36$, $p = 0.055$), suggesting a trend towards increased DJ-1 expression levels with age. According to the free radical theory of aging, the process of aging and its related diseases is associated with increased free-radical induced damage

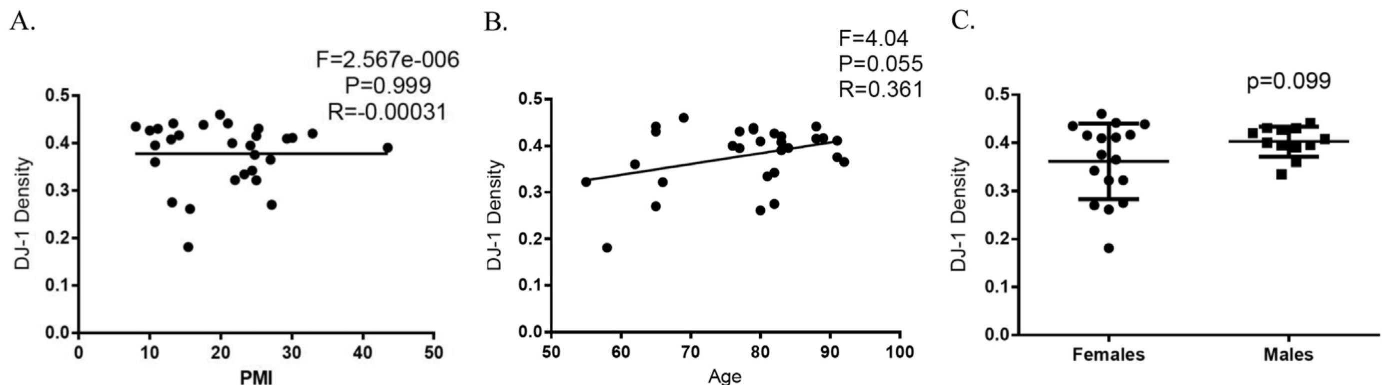

Fig. 4. Correlation between DJ-1 density and A. PMI and B. Age. Results were assessed using the Pearson's correlation test. C. The effect of sex was analyzed using the independent *t*-test. No significant difference between males and females. No significant correlations were found.

to biomolecules (Liochev, 2013). This theory is supported by increased oxidative damage to proteins and decreased levels of glutathione enzymes with age (Onorato et al., 1998; Jones et al., 2002). As age and oxidative stress are involved in various age-related diseases, this finding provides a possible explanation that underlies a trend towards increased DJ-1 expression levels with age. DJ-1 may be upregulated as a compensatory mechanism to offset the high oxidative stress in older BD subjects. Since DJ-1 might have the role of a redox sensor (Hayashi et al., 2009), and has been shown to directly regulate complex I activity to protect neurons against oxidative stress, it would therefore be interesting to assess DJ-1 expression levels in a larger cohort of older patients with BD.

Several notable limitations from our samples and methods may have limited our interpretation of the results. First, a major issue is inherent in the microarray technology itself, being differences in the designs and versions of the platforms used by investigators. Including gene expression studies that uses one specific platform would allow us to greatly normalize the analyses and significance threshold across all studies, therefore decreasing variability in the gene expression results. Secondly, the cross study analysis included microarray studies that used different regions of the frontal cortex (Brodman Area 46, 6 8/9, 10) and the cerebellum. Although including more brain regions in the analyses would lead to results that are generally more representative, it is possible that gene expression patterns in non-frontal and frontal regions may be different from each other, which might contribute to heterogeneity.

Post mortem brain samples also provide powerful insights of brain pathology via direct measure. However there are issues pertaining to its use leading to possible confounding variables such as brain pH and medications that could affect DJ-1 expression levels in post-mortem brains of BD subjects. Medications, particularly antipsychotics are known to impede electron transfer activity at complex I (Maurer and Moller, 1997; Balijepalli et al., 2001), however it is currently unknown whether it could alter regulation of complex I activity by modulating DJ-1 expression levels. Since these clinical descriptions of our samples were not provided, we cannot disregard the probability that these unobserved variables might affect DJ-1 expression profiles. Furthermore, the measure of fluorescence densities from our sections is only a relative measure because it is limited by the quality of brain tissue. However, in order to account for this, sections with good tissue quality were used and background densities were reduced. Since BD is a biphasic disorder, and we hypothesize that state dependent increases or decreases in mitochondrial activity might reflect mania and depression respectively, it is likely necessary in futures studies to assay material collected and stratify for mood state. This is not possible in a postmortem cohort.

5. Conclusion

To conclude, our hypothesis of the presence of altered complex I regulators based on the literature search was not supported by the microarray studies and our immunohistochemical validation data. Among the four genes (*PARK-7 STAT3, SIRT-3, and IMP2*), *PARK-7* showed a near consistent trend towards downregulation across individual microarray studies. However, this was not supported by our immunohistochemistry data, confirming that DJ-1 is not significantly differentially regulated in BD subjects. Furthermore, there was a trend towards increased DJ-1 expression levels with age in our correlation analysis, suggesting a possible role of DJ-1 in protecting against oxidative stress in older patients with BD. As mitochondrial complex I dysfunction is one of the most consistent findings in BD, it would therefore be valuable to further examine alterations in complex I regulators by other regulators. This would either result in non-confirmation of the hypothesis or prompt new hypotheses that unites regulatory mechanisms and BD development, which could provide new avenues for targeted therapy (Callaly et al., 2015).

Conflicts of interest

None.

Acknowledgments

We thank the Stanley Medical Research Institute (SMRI)–Online Genomics Database and Elashoff Consulting, LLC for providing access to the public available microarray data. We also thank Dr. Sabine Bahn, Dr. Tadafumi Kato, Dr. Marquis P. Vawter, Dr. Trevor Young, Dr. Seth E. Dobrin, Dr. Haiming Chen, Dr. Allen A. Fienberg, Dr. Pamela Sklar collaborators from SMRI Online Genomics Database. MB is supported by a NHMRC Senior Principal Research Fellowship 1059660. ACA is supported by Canadian Institutes of Health Research (CIHR 133439), Ontario Mental Health Foundation (OMHF 498567) and Ministry of Research and Innovation of Canada. LTY is supported by CIHR 133439.

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Curr Treat Options Psych (2016) 3:315–329
DOI 10.1007/s40501-016-0093-6

Mood Disorders (S Frangou, Section Editor)

Neurobiology of Risk for Bipolar Disorder

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Published online: 20 October 2016
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This article is part of the Topical Collection on *Mood Disorders*

Keywords Bipolar disorder · Endophenotype · Risk · Relatives · Neurocognition · Brain imaging · Oxidative stress

Abbreviations *BD* Bipolar disorder · *BDNF* Brain-derived neurotrophic factor · *BD-P* Bipolar disorder probands · *fMRI* Functional magnetic resonance imaging · *GM* Gray matter · *IL-6* Interleukin 6 · *8-OHdG* 8-Hydroxy-2′-deoxyguanosine · *ToM* Theory of mind · *WM* White matter

Opinion statement

Bipolar disorder (BD) is a chronic mental illness which follows a relapsing and remitting course and requires lifetime treatment. The lack of biological markers for BD is a major difficulty in clinical practice. Exploring multiple endophenotypes to fit in multivariate genetic models for BD is an important element in the process of finding tools to facilitate early diagnosis, early intervention, prevention of new episodes, and follow-up of treatment response in BD. Reviewing of studies on neuroimaging, neurocognition, and biochemical parameters in populations with high genetic risk for the illness can yield an integrative perspective on the neurobiology of risk for BD. The most up-to-date data reveals consistent deficits in executive function, response inhibition, verbal memory/learning, verbal fluency, and processing speed in risk groups for BD. Functional magnetic resonance imaging (fMRI) studies report alterations in the activity of the inferior frontal gyrus, medial prefrontal cortex, and limbic areas, particularly in the amygdala in unaffected first-degree relatives (FDR) of BD compared to healthy controls. Risk groups for BD also present altered immune and neurochemical modulation. Despite inconsistencies, accumulating data reveals cognitive and imaging markers for risk and to a less extent resilience of BD. Findings on neural modulation markers are preliminary and require further studies. Although the

knowledge on the neurobiology of risk for BD has been inadequate to provide benefits for clinical practice, further studies on structural and functional changes in the brain, neurocognitive functioning, and neurochemical modulation have a potential to reveal biomarkers for risk and resilience for BD. Multimodal, multicenter, population-based studies with large sample size allowing for homogeneous subgroup analyses will immensely contribute to the elucidation of biological markers for risk for BD in an integrative model.

Introduction

Bipolar disorder (BD) is a chronic mental illness which follows a relapsing and remitting course and requires lifetime treatment. In nearly two thirds of patients, the illness begins before the end of the third decade of life [1]. BD is heritable as shown by varying (59–93 %) yet high heritability rates [2, 3]. Concordance rates increase substantially from 6 % in dizygotic twins to 43 % in monozygotic twins [4]. Children of parents with BD are four times more likely to develop an affective disorder compared to children of parents with no mental disorders [5]. Delayed diagnosis and misdiagnosis are common in BD [6]. Despite evidence for substantial genetic load in the etiology of BD, clinical practice still suffers from the absence of biological markers which could be used in support of the clinical diagnosis.

Endophenotypes are an important subtype of biomarkers that have a clear genetic connection and are more prevalent in patients and in their family members [7]. Exploring multiple endophenotypes to fit in multivariate genetic models for bipolar disorder is an important element in the process of finding diagnostic tools to facilitate early intervention and prevention in BD.

In this review, we aimed to identify common neurocognitive, neuroanatomical, and neurochemical abnormalities that may correspond to vulnerability and resilience factors for BD. Literature on neuroimaging, neurocognition, and biochemical parameters in BD, particularly in populations with high genetic risk for the illness, was reviewed to shed light on the neurobiology of risk for bipolar disorder from an integrative perspective.

Method

Literature review was completed using keywords “bipolar disorder,” “endophenotype,” “risk,” “relatives,” “neurocognition,” “brain imaging,” and “oxidative stress.” Publications were searched using PubMed, Scopus, Science Direct, and Web of Science electronic databases. Papers published in English, which involved first-degree relatives (FDR-offspring, sibling, co-twins, parents) of a bipolar proband (BD-P) and a healthy control group with or without a patient group, were included. Publications with FDR of schizophrenia probands (SCH-P) in addition to FDR of BD-P, papers on studies modeling for a genetic link to a proposed biological marker, meta-analysis, and systematic reviews were also included. In each respective targeted area, individual studies published after the most recent meta-analysis and/or systematic review were included in the review. This article does not publish original research, animal or human studies, that would need informed consent that should be carried out by the authors.

Results

Neurocognition and risk for BD

Genetic influence on measures of various neurocognitive domains has been well documented [8]. Verbal ability, executive functioning, and psychomotor processing speed were shown to be highly heritable in familial BD [9]. A large-scale extended pedigree study suggested impaired processing speed, working memory, and declarative (facial) memory to be candidate endophenotypes for BD [10]. After controlling for demography and current mood symptoms, processing speed was still impaired in BD-P type I and their unaffected FDR, showing its validity as endophenotype to separate BD-P and FDR from healthy controls [11].

In search for potential cognitive endophenotypes, a systematic review and meta-analysis of data from studies on FDR (with or without BD-P) in comparison to healthy controls showed impaired executive function, verbal memory, and verbal working memory [12, 13]. Among executive functions, response inhibition deficits were the most robust candidates followed by impaired verbal memory, sustained attention, and set shifting even after controlling for IQ and age [14].

More recent studies focusing on healthy adolescent offsprings of parents with BD found that young FDR have impairments in processing speed and visual memory [15], cognitive flexibility [16], psychomotor speed, focused attention, verbal attention, phonemic verbal fluency, short-term memory and learning [17], verbal intelligence [18], and significantly slower reaction times on an index of executive attention [19] compared to youth with healthy parents. Likewise, healthy parents of patients with BD-I had significantly worse performance in psychomotor speed, cognitive flexibility, selective attention, response inhibition, and verbal memory [20, 21] than healthy controls. A recent review of conscript, cohort, high-risk, family-based and first-episode mania studies also confirmed that verbal memory and executive function are potential predictors of BD [22].

Recent studies, however, provide further more nuanced evidence specifically with regard to impaired response inhibition and interference control in both adult [23] and adolescent BD-P [24] and their FDR compared to healthy controls. Other studies found that response inhibition was intact despite increased impulsivity and impulsive decision-making in both familial and nonfamilial high-risk groups for BD [25] and interference control was intact in FDR and co-twins of BD-P [26]. Evidence shows significantly worse response inhibition performance in BD-P I with history of psychotic symptoms and their FDR compared to controls [27]. Response inhibition deficit was associated with the process of illness with psychotic features in BD, rather than being a vulnerability marker [28]. On the other hand, impulsivity as measured by BIS-11 (a self-report scale) seems to reveal more consistent signals as a candidate endophenotype both in children, adolescents [29], and adults [25, 30, 31], and as a predictor of onset of BD in reward-sensitive adolescents and young adults [32]. However, specificity of impulsivity to BD is questionable as it shows shared genetic liability with SCH and major depressive disorder [30] and requires further studies. Risk-taking behavior may also be a potential endophenotype and predictor of BD [32, 33].

Recent studies focusing on facial emotion recognition and emotional responsiveness in at-risk relatives compared to healthy controls showed deficits in labeling facial emotion, required significantly more time and more intense emotional information to identify and correctly label face emotions, and were impaired in other aspects of affective response particularly in inhibiting negative valenced stimuli and in having greater response bias toward negatively valenced stimuli [18, 34–39]. Social cognition is another recent area of interest in defining endophenotypes for BD for which theory of mind (ToM) performance has been most commonly considered. BD-FDR performed significantly worse on the verbal but not visual or higher-order ToM tasks compared to healthy controls [40]; their performance was comparable to healthy controls on tasks requiring ToM use and ToM understanding [41].

Do cognitive deficits exist before onset of illness in BD?

Systematic review of data from 23 studies on the premorbid cognitive function of people who later developed BD and of BD-P when presenting with their first episode provided evidence that general intelligence is not impaired in the premorbid stage; however, verbal memory, attention, and executive function deficits tend to be present during and after the first episode. Data supports the notion that specific cognitive domain deficits may precede the illness onset in BD [42•]. However, assessment of premorbid intellectual in BD function may yield contradictory findings, depending on whether the assessment is retrospective or prospective [43].

Are the neurocognitive markers specific to BD? Role of psychosis

The genetic etiology of BD and SCH overlap substantially [2]. Enhanced susceptibility to interference and reduced inhibition [44] as well as deficits in working memory were reported to be more common in BD patients with psychosis, in SCH patients, and their FDR compared to healthy controls [45]. Severity of premorbid intellectual deficit differs quantitatively between BD and SCH. BD presents significant yet small premorbid intellectual function deficits when assessed retrospectively but not prospectively and moderate cognitive impairment after onset of illness, whereas SCH presents with significant premorbid and large post-onset impairment [43]. It appears that both disorders are associated with impaired visual sustained attention which does not differentiate one condition from another [46].

Further differences between BD and SCH have been observed when examining the association between single nucleotide polymorphisms (SNP) in key risk genes in connection to cognitive tests which are closely linked to prefrontal cortical functioning. The Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene is associated with performance on the Wisconsin Cart Sorting Test in BD but not in SCH, while the reverse is the case for task performance on the N-back working memory task [47]. Among previously identified candidate sets of genes associated with cognitive abilities in SCH or BD visuospatial attention, verbal abilities sets and delayed verbal memory showed the strongest enrichments in BD, whereas color-word interference (cognitive

inhibition) test and sets associated with memory learning slope showed enrichment in SCH [48].

Testing SCH-FDR and BD-FDR in comparison to healthy controls on Stroop Color-Word Task and Emotional Stroop Task showed impaired cognitive inhibition in SCH-FDR but emotional bias toward mood-related information in BD-FDR [49]. Assessment of executive functions by using Wisconsin Cart Sorting Test and part A and part B of the Trail Making Task in SCH, BD, and FDR of both groups revealed familial resemblance for both tests in BD families, whereas no resemblance was observed in families with SCH [50]. Using psychosis as a dimension in grouping the participants, a family study revealed a gradient of performance on the working and declarative memory, executive functions, and attention with the poorest being in probands (i.e., SCH-P, BD-P with psychosis), intermediate in FDR of the psychosis spectrum, and highest in the FDR of the nonpsychotic spectrum disorder, supporting the notion that cognitive function in BD and SCH defines a psychosis continuum [51]. Structural equation modeling of cognitive data from 331 twins/siblings showed that illness state and concordance for BD had a modest impact of verbal episodic memory and spatial working memory on the bipolar diathesis; IQ and visual-spatial learning, however, were associated with genetic diathesis to BD and with nonaffective symptomatology, also supporting the notion of psychosis continuum [52].

In an extensive review of studies investigating neurocognitive deficits in premorbid, high-risk and first-episode BD in comparison to outcome studies in SCH, Bora proposed a model where only BD-P who are prone to psychosis may show premorbid neurodevelopmental cognitive deficits similar to SCH. In the absence of psychosis and neurodevelopmental deficits, BD-associated temperamental characteristics set the stage between supranormal premorbid cognition and risk for BP [53••]. Examination of the cognitive profiles of at-risk individuals for BD and BD-P did not appear to support previous suggestions of progressive cognitive decline in BD with illness development [18].

In summary, deficits in executive function, response inhibition, verbal memory/learning, verbal fluency, processing speed, and verbal fluency seem to be promising cognitive markers for risk of developing BD. However, there are limitations in the literature related to the variability of the tests used in measuring the same cognitive domains by different groups, inclusion of varying age groups, nonstandardized definition and use of mixed groups of at-risk individuals (i.e., offspring, siblings, parents), small sample size, and not accounting for the presence of history of psychosis. Such methodological issues cause inconsistencies in the findings and difficulty in interpreting the corresponding functional deficits. Data are still limited on the presence and pattern of premorbid cognitive impairment in the risk population. Findings obtained from cross-sectional studies without controlling for premorbid cognitive impairment may exaggerate the magnitude and misidentify the type of cognitive deficits to be used as markers for risk of BD. Although it may not be specific to BD, the effect of deficits in processing speed on other test performance in patients and to a less extend in the risk groups and controls [54] should be taken into consideration.

Brain imaging and risk for BD

Structural imaging findings

Gray matter (GM) abnormalities

In a recent meta-analysis of data from structural and functional imaging studies, the GM volume of individuals at risk for BD did not differ significantly from healthy controls, including regions traditionally associated with BD, such as the striatum, thalamus, amygdala, hippocampus, and pituitary. The results of this meta-analysis challenge the notion that brain morphology can yield endophenotypic markers for BD. The authors also capitalize on the susceptibility of the hippocampus to nongenetic/ environmental factors as obstetric complications and stress-induced excessive glucocorticoid exposure. They also draw attention to an association between inconsistent pituitary findings and state-dependent cortisol abnormalities in mood disorders [55]. Assessment of dexamethasone-suppression-CRH test in high-risk individuals who developed an affective disorder in a 10-year follow-up period revealed no premorbid differences in their cortisol response compared to healthy controls [56]. Dysregulated hypothalamo-pituitary-adrenal (HPA) axis abnormalities in BD can be regarded as a neurobiological scar developing during the course of affective disorders rather than a neuroendocrine vulnerability marker [56]. A later review on studies investigating cortical or subcortical GM abnormalities in BD-FDR shows that findings on various brain regions across studies are inconsistent except for larger insular cortex volumes in adult first-degree relatives and larger right inferior frontal gyrus in BD offspring, in comparison to healthy controls [57].

Recent studies support the above findings with larger inferior frontal gyrus, left insula, smaller cerebellar, and left orbitofrontal gyrus GM volumes being shared both in BD-P and their FDR [58–60], and larger parahippocampal and left dorsolateral prefrontal cortex appeared only in BD-FDR [58, 59].

It is worth remembering that the inferior frontal gyrus has a pivotal role in response inhibition and emotion regulation, both of which have been suggested as candidate endophenotypes for BD whereas the cerebellum has extensive connections to brain areas that are involved in cognition and behavior including the prefrontal cortex, anterior cingulate, and limbic system through cortico-ponto-cerebellar and cerebello-thalamo-cortical pathways. The cerebellum also has a homeostatic role in affect regulation in addition to motor functions [59].

Despite an association between genetic liability for BD and GM volumes in regions of the anterior cingulate cortex, ventral striatum, medial frontal gyrus, right precentral gyrus, right insular cortex, and medial orbital gyrus [57], the absence of evidence for GM abnormalities and contradictory findings [61, 62] across studies in BD-FDR may be due to nongenetic factors such as age, clinical features, medication, duration illness, pubertal stage, and age of onset [63–67]. Increased GM volume or thickness of these regions may be consequent of neuroprotective compensatory mechanisms or abnormal brain

maturation due to the maladaptive pruning in at-risk group or may be associated with resilience [59].

The search of a relationship between neuroanatomical changes and genetic risk for BD or SCH showed a specific association between SCH and distributed GM volume loss in the bilateral fronto-striato-thalamic and left lateral temporal regions and enlarged lateral ventricles; genetic risk for BD was specifically associated with GM deficits in the right anterior cingulate gyrus and ventral striatum [55].

White matter (WM) abnormalities

There is a limited number of diffusion tensor imaging (DTI) studies of unaffected FDR of BD-P. Some studies found abnormalities in the superior longitudinal fasciculus, inferior longitudinal fasciculus, corpus callosum, right uncinate fasciculus, right inferior fronto-occipital fasciculus, right anterior limb of internal capsule, and thalamic radiation in both BD-P and BD-FDR [68•, 69], while two studies did not find any abnormalities in older relative groups compared with controls [70, 71]. A population-based study showed abnormalities in similar white matter tracts in adolescents with subthreshold bipolar symptoms [72]. These tracts connect regions implicated in the identification and regulation of emotion, attention, impulsivity, response inhibition, set shifting, and risk-taking [68•]. Decreased WM volume is highly associated with genetic risk and familiality in BD [70–74]. However, the results are neither consistent nor robust enough to indicate replicable WM abnormalities in BD-FDR, thus not supportive of the WM abnormalities as an endophenotype of BD, whereas more WM abnormalities in SCH conform better to the concept of endophenotype [68•, 73].

Functional neuroimaging

Recent years have witnessed the publication of a sizeable number of task and resting state fMRI in BD-P and their FDR in comparison to healthy controls.

Resting state

Resting state connectivity between the frontal cortex and basal ganglia or limbic/paralimbic regions was shown to be altered in a nonspecific fashion in unaffected BD-FDR [75•]. Solé-Padullés et al. found no connection differences between any regions in BD-FDR compared with SCH-FDR and healthy controls [76].

Cognitive task—fMRI studies

When reviewing the relevant studies, we found that patients and their parents show activation differences in the frontal cortex, insula, amygdala, parietal cortex, and cingulate cortex as well as connectivity defects between these regions [55, 75•]. Hyperactivation of inferior frontal gyrus (including ventrolateral prefrontal cortex and orbitofrontal cortex) and hypoactivation of insula, amygdala, basal ganglions, and limbic system, which were

signified in at-risk group, are interpreted as compensatory mechanisms [75•, 77, 78]. An fMRI study found differential effects of the DISC1 Leu607Phe polymorphism on the left pre/postcentral gyrus, extending to inferior frontal gyrus in FDR of BD and SCH during language task [79]. However, relatively small sample sizes of the studies limit the generalizability of the findings.

Emotional task—fMRI studies

Studies which investigated neural substrate of emotional dysregulation in risk groups for BD showed altered activity in insula and ventrolateral prefrontal cortex, dysfunctional connectivity in orbitofrontal cortex-amygdala, and impairment in downregulation of amygdala [75•, 77, 80–83]. Breakspear et al. showed impaired hierarchical model (dorsolateral prefrontal cortex-inferior frontal gyrus-anterior cingulate cortex) and reduced activity of inferior frontal gyrus in BD relatives [84]. In the risk group, the change produced by the negative affect in the brain regions was more evident than the positive affect [81, 83]. This is an important finding, yielding a new research area in the light of the fact that response to positive affect is more sensitive to environmental factors and that it could easily be lost compared to the control group [81, 83].

In summary, fMRI studies present alterations in the activity of the same regions involved in the pathophysiology of BD, namely the inferior frontal gyrus, medial prefrontal cortex, and limbic area particularly in the amygdala, in unaffected BD-FDR, in comparison to healthy controls.

Genetics and white matter neuroimaging in the risk for BD

Studies combining genetics and neuroimaging demonstrated association between decreased WM in BD-FDR and disrupted NRG1-ErbB4, calcium signaling (CACNA1C), phosphatidylinositol, and CAMs pathways [74, 85]. These pathways relate to WM development, neuronal plasticity, regulation of neurotransmitter release, and cell adhesion [74]. Another study reported an association between FA reduction in the WM tracts which are involved in the pathophysiology of BD and higher polygenic risk scores in affected but not in unaffected relatives [86], which suggest that WM abnormalities are closely linked to expression of psychopathology rather than genetic risk per se.

Overall, there are discrepancies in the results from both structural and functional imaging studies despite promising findings identifying risk markers for BD. Different imaging techniques that had been applied, heterogeneous clinical and demographical profiles of the participants, small sample size, variability of the tasks, heterogeneity of the definition of the risk groups (offspring vs. siblings vs. parents, vs. mixed group of FDR), presence of subsyndromal symptoms in the risk groups in some studies as well as variability in age of the participants contribute to difficulties in identifying a specific pattern of alterations for individuals at risk. Also, the effects of environmental factors and the association between clinical features and MRI findings are not well known. The absence of fMRI studies investigating social cognition, risk-taking, and response inhibition, is also noteworthy.

Inflammation, oxidation, neurotrophins, and other mediators and risk for BD

Exploration of the inflammatory processes on the neuronal function of risk groups is important for a better understanding of the molecular basis of risk for BD as accumulating data implicate these processes in the pathogenesis of BD.

Among several molecules which are suggested to be involved in the pathogenesis of BD and are known to be involved in inflammation (interleukin 1, interleukin 6 (IL-6), interleukin 10, interleukin 17, interferon gamma), oxidative stress (thiobarbituric acid reactive substances, protein carbonyl content), and neurotrophins (BDNF), only IL-6 levels have been found to be significantly higher in BD-P compared to BD-FDR [87]. However, increased IL-6 and BDNF plasma levels have been reported in BD offspring compared with healthy controls. High-risk offspring that appeared to have prodromal symptoms presented with higher plasma levels of IL-6 and BDNF than high-risk offspring that appeared asymptomatic or mildly symptomatic [88].

In a prospective follow-up, BD offspring showed increased proinflammatory gene expression in monocytes during adolescence, but not in adulthood [89]. Specifically, in that study, BD offspring had persistent monocyte activation during adolescence and early adulthood as shown by increased cytokine pentraxin-related protein (PTX3) levels and T-regulatory cells and decreased effector T cells (Th1 and Th17). Despite decreased serum levels of BDNF, normal levels of chemokine (C-C motif) ligand 2 (CCL2), and S100 calcium-binding protein B (S100B) during adolescence, BD offspring showed increased levels of CCL2, BDNF, and S100B in adulthood [89]. These findings suggest an abnormal neuroimmune state in BD offspring, which followed a dynamic course from adolescence into adulthood.

Most recently, plasma levels of lipid peroxidation (lipid hydroperoxide and 4-hydroxy-2-nonenal, 8-isoprostane), protein oxidation (protein carbonyls), and inflammation (interleukin 1, interleukin 6, interleukin 10, interferon gamma, TNF alfa) were assessed in four groups of adolescents (9–20 years of age), consisting of high-risk offspring, ultrahigh-risk offspring, first-episode BD patients, and healthy controls [90]. The levels of lipid hydroperoxide, an early stage lipid peroxidation marker, showed a decreasing trend along the spectrum of risk for BD-I, while there was no difference in the late stage lipid peroxidation markers (4-hydroxy-2-nonenal, 8-isoprostane), protein carbonyls, and inflammatory markers among groups [90].

Serum BDNF levels were found to be decreased [91] or unchanged [92, 93] in BD-P and BD-FDR compared to healthy controls. Duffy et al. reported that the BDNF genotype significantly moderates the association between high-risk status for both gene expression and protein levels in BD offspring [88]. Correspondingly, anxiety symptoms were associated with the BDNF risk genotype only in BD offspring but not in healthy controls, and BD offspring with the val/val genotype showed higher anxiety symptoms than BD offspring with other genotypes [94].

Ferensztajn et al. reported higher BDNF and matrix metalloproteinase-9 levels and lower IL-6 levels in the offspring of BD-P who were excellent lithium responders compared to the offspring of BD-P who were lithium nonresponders [95].

Comparison of biomarkers related to oxidative stress [8-hydroxy-2′-deoxyguanosine (8-OHdG), mitochondrial complex 1 activation, and glutathione peroxidase activities] and global DNA methylation (5-

methylcytosine) between lithium responder BD-P, BD-FDR and healthy controls showed that BD-FDR have decreased global methylation, increased glutathione peroxidase activity, and no change in 8-OHdG or in mitochondrial complex 1 activity [96].

These results show that risk groups for BD present with altered immune and neurochemical modulation. However, the findings are preliminary, and studies on well-defined and clinically homogeneous risk groups, particularly in prospective design to understand the risk and defense mechanisms, are needed.

Conclusion and future directions

Prospective long-term follow-up studies using multimodal (i.e., combination of imaging, cognitive, neurochemical, and genetic assessment of the participants) and standardized techniques (i.e., the same set of cognitive tasks per domain) in well-defined at-risk populations, controlling for age and gender distribution as well as for the presence of symptoms, are needed for better understanding of the neurobiology of risk for BD [97]. Operationalized criteria for defining risk and resilience markers would also assist in improving our understanding of the complex changes observed in patients and their relatives. This would also foster further research into disambiguating compensatory from pathological processes. It is unclear whether disease-specific biomarkers can indeed be identified as most indications point to significant overlap between disorders which has generally motivated a trans-diagnostic approach to psychiatric research. Another unmet need is the information on interaction between immune and neurochemical alterations and cognitive and structural/functional changes. Studies exploring the associations between neurochemical, cognitive, and imaging are needed. Multicenter, population-based studies with large sample size allowing for homogeneous subgroup analysis (i.e., relatives of BD type I vs. type II, psychotic vs. nonpsychotic, offspring vs. sibling, symptomatic vs. asymptomatic, adolescent vs. adult; treatment responder vs. nonresponder) searching for cognitive, imaging, and neurochemical modulatory markers for risk for BD in an integrative way will immensely contribute to the field.

Highlights of the review

The review includes data on cognitive functions, structural and functional imaging, and neurochemical modulation as potential markers for risk and resilience for BD in an integrative way. The most recent data in each respective field has been included in the review besides meta-analysis and systematic reviews.

Despite inconsistencies, compiling data reveals cognitive and imaging markers for risk and to a less extent resilience of BD. Findings on neural modulation markers are preliminary and require further studies. Methodological issues causing obstacle in interpretation of the existing data have been considered.

Acknowledgments

The authors thank "Lithium Association" as the funding source of the presented review.

Compliance with Ethical Standards

Conflict of Interest
Dr. Özerdem and Dr. Ceylan receive research support from the Scientific and Technological Research Council of Turkey (TUBITAK). Dr. Özerdem and Dr. Can receive research support from the Dokuz Eylül University Scientific Research Projects Coordination Unit.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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Current Drug Metabolism, **2018**, *19*, 000-000

REVIEW ARTICLE

The Relationship Between Lithium and Cancer Proliferation: A Case-Based Review of the Literature

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ARTICLE HISTORY

Received: March 24, 2017
Revised: December 18, 2017
Accepted: March 28, 2018

DOI:
10.2174/1389200219666180430095933

Abstract: ***Background:*** The incidence and mortality rates of cancer in patients with Bipolar Disorder (BD) is higher compared with the general population. The role of Lithium (Li) in cancer proliferation/inhibition is still a controversial issue in the literature.

Objective: Based on a clinical case with lithium intake and development of a renal tumor, we aimed to explore the relationship between Li use and tumor proliferation, with regard to the mechanism of action of Li.

Method: We present evidence of a female patient with bipolar disorder I, who had been on Li for several years, either as monotherapy or in combination with Valproate (VPA). While on Li monotherapy, the patient had undergone unilateral nephrectomy due to a chromophobe cell renal tumor. A literature search was performed using keywords "bipolar disorder, medical comorbidity, cancer, renal tumor, lithium, mood stabilizers, valproate and mechanism of action."

Results: The limited data on the relationship between Li and cancer proliferation in clinical populations support neither a positive relationship between long-term Li use and increased urinary tract cancers nor an overall cancer risk. Growing evidence identifies effects of Li on cancer proliferation through inhibition of glycogen synthase kinase-3β (GSK-3β), modulations of redox status, inflammatory changes, pro-/anti-apoptotic mechanisms, and mitochondrial function changes.

Conclusion: Despite the presence of contradictory data, a substantial body of evidence mainly from molecular studies points to Li's anti-carcinogenic effects. However, the underlying mechanistic pathways remain unclear. Mitochondrial dysfunction and redox modulations are potential areas for research on the relationship between Li and cancer proliferation.

Keywords: Lithium, cancer proliferation, urinary tract cancer, tumor, GSK-3, redox modulations, mitochondrial damage.

1. INTRODUCTION

Bipolar disorder is a severe mental illness associated with significant morbidity and mortality [1-3]. It is characterized by frequent and recurrent episodes of depression, mania and or hypomania. [4]. Bipolar disorder affects 1% to 3% of the general population with an onset mainly in adolescence or early adulthood [5]. Patients with bipolar disorder, regardless of treatment type, constitute a risk group for various medical comorbidities [3]. Compared to the community, patients with bipolar disorder were found to present increased risk and mortality rates due to cancer [6, 7]. However, in a population-based study, the overall cancer risk was found to be similar between patients with bipolar disorder and the community in general [8].

Carbonate salt of Li has been used for patients with bipolar disorder for more than 50 years and is still considered to be the standard medication in the pharmacological management of bipolar disorder [9]. It is the first line treatment choice for all phases of bipolar disorder in several treatment guidelines [10-13]. However, a growing body of evidence suggests that the effects of Li extend beyond these clinical outcomes through its mode of action, by modulating several homeostatic mechanisms, including the pathways involved in cancer proliferation [14].

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A wide variety of *in-vivo* and *in vitro* studies showed that Li inhibits the proliferation of various types of cancer; such as glioma [14], medulloblastoma [15], colorectal [16], hepatocellular [17] breast [18] and ovarian [19]. However, there is conflicting data on the overall cancer risk concerning the use of Li in bipolar disorder. In the previously mentioned population-based study [8], despite a similar overall cancer risk between bipolar disorder patients and the general population, increased cancer risk in the gastrointestinal, respiratory and endocrine systems was found in patients without lithium treatment, compared to patients with lithium treatment, who did not present any significant increase in risk of cancer compared to general population [8]. Another population-based study showed an association between lithium exposure and significantly lower cancer risk compared with anticonvulsant-only exposure [20]. In contrast, some publications suggested an increase in cancer proliferation was caused by long-term Li use [21-23].

Here we present the case of a female patient who had lived with bipolar disorder type I for the last 26 years and had a recent unilateral nephrectomy due to a renal tumor. Due to lithium's effectiveness in the maintenance treatment, and patient's willingness to use Li before and after the surgical removal of the tumor, there was a need to explore the relationship between tumor proliferation and Li, and risk/benefit assessment for patient's future treatment. The patient was informed about the case report, and she consented to it. A literature search used keywords "bipolar disorder and medical comorbidity, and cancer, and renal tumor, and lithium, and mood stabilizers, and valproate, and mechanism of action." This review

1389-2002/18 $58.00+.00 © 2018 Bentham Science Publishers

covers the relationship between Li use and cancer, particularly focusing on the drug's mechanisms of action including effects on the GSK-3 system, redox modulation, and mitochondrial improvement. A potential interference of valproate on the cancer development is also discussed within the context of its mechanism of action, as valproate had been used for maintenance as monotherapy or in combination with Li during the course of the illness in the presented case.

2. CASE REPORT

The patient is a 46-year-old female high school graduate, a housewife who lives with her husband and daughter (Fig.1). The patient did not have a known medical comorbidity. She has been a smoker for the past 30 years (one pack of cigarettes/day).

The patient's illness started at age 20 with a depressive episode, for which she prescribed on antidepressant treatment. The episode ended with a manic switch. She was hospitalized and treated successfully with ECT. Li monotherapy was initiated for maintenance after remission. Between the years 1990-2002, she remained stable on Li monotherapy, and during this period she married and had a child. Only while pregnancy, she stopped taking Li. The patient was hospitalized for an acute manic episode in 2002, in spite of her ongoing regular Li monotherapy with therapeutic serum levels. Li was discontinued on the assumption that it was no longer successful in preventing mood episodes. The manic episode remitted on Valproate (VPA) monotherapy. Since then, the patient has one mild, and one moderately severe depressive episode, in 2004 and 2006 respectively while on VPA monotherapy with therapeutic serum levels. The mild depression recovered with VPA alone. However, quetiapine was added to VPA for the depressive episode in 2006, after a trial of lamotrigine, was abandoned as it caused a skin rash. During a regular medical examination for her ongoing follow-up visits, a renal mass (angiomyolipoma) was explored coincidently with abdominal ultrasonography (USG) examination in 2006. From there on, the patient was followed up by both psychiatry and nephrology outpatient units. She continued to be stable on VPA at therapeutic serum levels (50-100 ug/mL) in combination with quetiapine (200 mg/day) for three years. After doses of both medications were lowered in 2009, she experienced a severe depressive episode and attempted suicide by taking high doses of multiple psychotropic medications. As a result, the patient was hospitalized, and remission of the episode was achieved with a combination of Li, VPA, and quetiapine. Both Li and VPA were at therapeutic serum levels, and the quetiapine doses were between 300-600 mg/day. Within weeks of remission, quetiapine was tapered and finally removed from the treatment. The patient remained euthymic, fully functional on Li and VPA combination therapy until 2013; however, due to severe hair loss and patient's non-compliance with the drug, VPA was discontinued. In 2014, during Li prophylaxis, as a result of symptoms of irritability, decreased sleep and anger attacks, olanzapine (5 mg/day) was added to the treatment for two months, then withdrawn upon successful remission of symptoms. After the diagnosis of renal mass, between 2006 and 2016, patient's renal functions remained intact despite erythrocyturia and hemoglobinuria, and serum Li and VPA levels were always within the therapeutic range. The patient was followed up regularly both by the bipolar disorders and nephrology outpatient units with all necessary physical and laboratory examinations, particularly with regular USG exams. A rapid enlargement of the renal tumor was detected in the spring of 2016, and in May 2016 the tumor removal together with unilateral nephrectomy was performed. Pathological examination of the surgical specimen revealed the diagnosis of the chromophobe-cell renal tumor. The patient stayed off Li for only three days during the peri-operational period and returned immediately to her usual dose. However, the patient needed to use additional olanzapine (10 mg/day) for two weeks due to hypomanic symptoms which occurred soon after the operation. After remitting, she returned to Li monotherapy and her regular follow-up visits with two/three-month intervals for her psychiatric condition and her renal functions. Serum Li level remained at low therapeutic range (0.61-0.81 mmol/L) and renal functions were intact (BUN 8.9 mg/dL, creatinin 0.87 mg/dL), with a slightly low e-GFR (CKD-EPI). The patient was psychiatrically stable, with CGI Score 1 and GAF score between 90-100 on the last visit (10 months after nephrectomy).

3. WHAT ARE ANGIOMYOLIPOMA AND CHROMOPHOBE RENAL CELL CARCINOMA?

Angiomyolipoma is a benign tumor of the kidney which includes blood vessels, smooth muscle cells, and fat cells. It appears in 0.013% of the population as determined by USG [24]. One of the two types of renal angiomyolipoma develops unilaterally, in contrast to the first type, which is seen in patients with tuberous sclerosis. However, the two types are histologically similar. It is very rare that angiomyolipoma is confused with renal cell carcinoma [25]. The majority of angiomyolipomas stay asymptomatic until they reach 4 cm in diameter. The major risk is bleeding, for which removal or embolectomy becomes necessary [24, 26]. On rare occasions, angiomyolipoma can transform to malignancy of varying types. However, there are case reports of malignant transformation of angiomyolipomas with prominent nuclear pleomorphism [25, 27, 28].

Chromophobe renal cell carcinoma is a rare type of kidney cancer. It develops in the cells lining the small tubules of the kidney. The tumor is known to be related to the genetic disorder called Birt-Hogg-Dubé syndrome. However, there is also the sporadic non-hereditary type of renal cancer and the genetic basis of which is little understood. It is known to present enriched metabolic pathways that are related to energy production in mitochondria. TP53 and PTEN are the two well-established tumor suppressor genes that were found to be frequently mutated in chromophobe renal cell carcinoma [29]. The largest chromophobe cell renal carcinoma case series of 291 cases reported good prognosis, with 93% and 88.9% cancer-specific survival rates at 5- and 10-years respectively, and a low tendency to progress and metastasize. Patient gender appeared as an independent predictor of cancer-specific survival, with female patients having a significantly lower risk of dying [30].

4. LITHIUM AND CANCER: MOLECULAR MECHANISMS

Li has been shown to be involved in cancer proliferation by several mechanisms operating on pro-/anti-apoptotic proteins, several transcription factors and autophagy [31]. Some of these mechanisms are involved in cancer growth, while others in the suppression of cancer growth. Studies on androgen-independent human prostate cells and the prostate cancer stem cells showed that Li exerts concentration [32] and time [33] dependent biphasic effect on these cells. While stimulation with low concentrations of lithium led to high proliferation, low apoptotic indices, high differentiation growth factor levels and healthier ultrastructure, high concentrations led to the opposite effect [32]. In another study, as Li stayed longer in the system, the viability of the human prostate cells decreased significantly regardless of concentration [33]. Li may inhibit cancer proliferation through modulating redox balance and improving mitochondrial functions which constitute Li's anti-oxidative properties [34]. Growing evidence identifies inhibition of Glycogen Synthase Kinase-3 (GSK-3), modulations of redox, inflammatory, and pro-/anti-apoptotic processes as the mechanisms through which Li affects cancer proliferation [35]. Recent research focusing on the effects of Li on redox modulations and mitochondrial functions offer a novel insight into the effects of Li on cancer proliferation [31, 34-36].

5. LITHIUM MAY AFFECT CANCER PROLIFERATION THROUGH GSK-3B INHIBITION: ACTIVATOR OR SUPRESSOR?

The GSK-3 enzyme is composed of two isoforms, GSK-3α and GSK-3β. Studies on the relationship between Li and GSK-3 focus

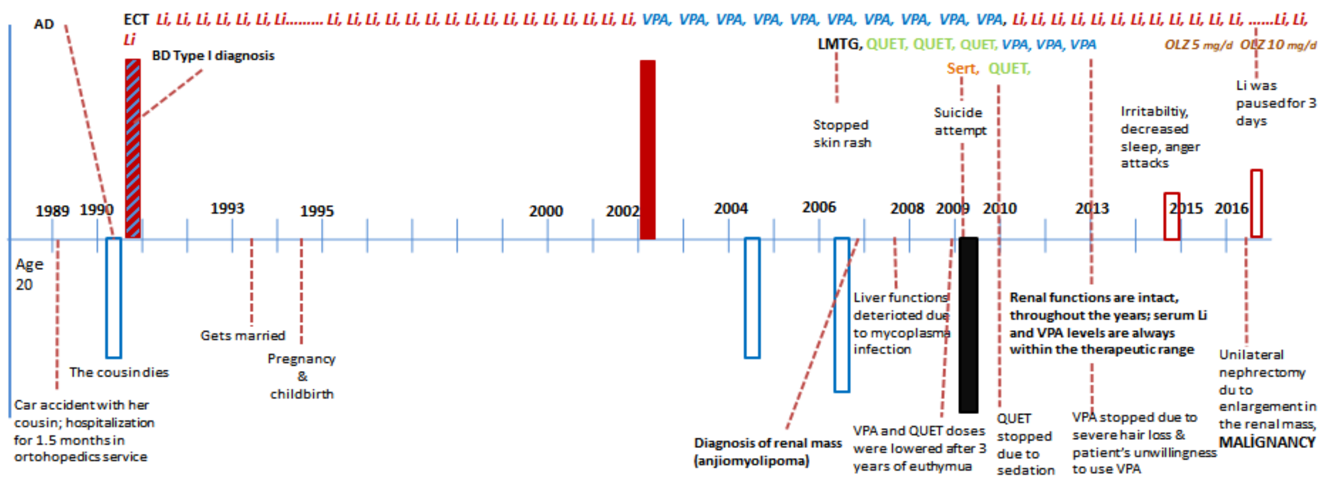

Fig. (1). Life chart of the patient. The horizontal line represents euthymia. Above it is the elevation, below is the depression state. Between each tic on the euthymia line is one year. The vertical line on the far left side and the tics on it represent the severity of mood episodes. As the tics get far from the euthymia line, the severity increases. The boxes above and below the euthymia line are the mood episodes. Manic episodes are in red color, depressive episodes are in blue color. Filled boxes represent hospitalization. Life events are given in the far below part of the chart. Far above part is reserved for medications.
AD: antidepressant; BD: bipolar disorder, Li: Lithium, VPA: Valproate; OLZ: Olanzapine; Sert: Sertraline; LMTG: Lamotrigine; QUET: Quetiapine.

either on GSK-3 in general or GSK-3β in particular. The inhibition of GSK-3 by Li has become the most extensively investigated pathway to illuminate the link between Li and cancer proliferation [37]. Li binds and inhibits GSK-3, a critical enzyme which coordinates multiple signaling pathways that regulate cellular processes including gene transcription, cell cycle progression, cell differentiation, survival and apoptosis [38, 39]. It does this by competing for a Mg2+ binding site and also increases the inhibitory serine phosphorylation of GSK-3 [40, 41]. GSK-3β has been linked to various medical disorders, psychiatric disorders including bipolar disorder, and also cancers [42-44]. The role of GSK-3 in general, and GSK-3β in particular, in cancer development, is controversial. There are disagreements over whether it promotes cell proliferation or acts as a tumor suppressor, and this complex process is still not completely understood [43, 44].

GSK-3 has been considered to be a tumor suppressor that inhibits its cellular tumor proliferation; thus GSK-3 inhibition by Li may activate cancer proliferation. GSK-3β inhibits several proto-oncogenes and cell cycle regulators through the Wnt/β-catenin and Hedgehog signaling pathways, two major pathways which are often dysregulated in cancers [45]. GSK-3 can phosphorylate and suppress the Wnt/ β-catenin pathway, which was shown to be aberrant in various types of cancers [46-50]. GSK-3 is involved in Hedgehog [Hh] signaling pathway, which is significantly linked to several cancer types, including skin [51], lung [52], breast [53], ovarian [54, 55], prostate [56] cancers. GSK-3 regulates specific components of Hh signaling pathway, such as activating transcription by phosphorylating SUFU, which is an inhibitor of Hh pathway [44]. Furthermore, GSK-3 has been suspected of contributing to cancer proliferation by inhibiting autophagy in cancer cells, through regulation of mammalian target of rapamycin [mTOR], a known player in malignant transformation [57].

In contrast, growing evidence suggests that GSK-3ß acts as an activator of cancer proliferation, and therefore GSK-3 inhibition by Li may suppress cancer proliferation. GSK-3ß protein was shown to be over-expressed in various types of cancers [57-59]. GSK-3 inhibition has been suggested to be a target in cancer treatment [44, 45, 60], because GSK-3ß inhibitors reduces cancer proliferation in various types of cancers, such as thyroid [61], prostate [62, 63], breast [64] and lung cancer [65], enhance the efficacy of the chemotherapy among resistant cancers [64, 66, 67]. Moreover, GSK-3 inhibition has been implicated in cancer management as a preventive treatment [68]. GSK-3ß modulates several anti-apoptotic pathways involved in nuclear factor-κB and facilitates NF-kB function [69-71], which has previously been linked to cancer progression, metastasis and chemotherapy resistance [72]. Consequently, it was demonstrated that GSK-3ß inhibition results in decreased expression of NF-kB target genes (such as Bcl-2, Mcl-1, and XIAP) and cell survival of cancer cells [69, 70]. GSK-3 also affects pro-apoptotic BCL-2 family, and GSK-3ß inhibition leads to increased expression of pro-apoptotic Bim in pancreatic cancer cells [73]. GSK-3ß induced apoptosis in neuronal cells, through stabilization of mitochondrial BAX by phosphorylation [74]. Additionally, there is evidence that repression of RNA polymerase 1 transcription by GSK3ß contributes to its tumor suppressor action [75].

Li and Pro-/Anti-Apoptotic Proteins, Several Transcription Factors and Autophagy

BCL-2 gene family members are key regulators of apoptotic cell death. Included in this gene family are both pro-apoptotic genes, such as BAX and BAK1, and anti-apoptotic genes, such as BCL-2 and BCL-xL [76]. Several preclinical studies have shown that Li increases Bcl-2 expression [77-82]. This particular effect of Li has been closely associated with its cytoprotective effects [77-84]. Li has been demonstrated to inhibit Bcl-2 dephosphorylation, which is required for the full anti-apoptotic functioning of Bcl-2 [85]. Furthermore, a clinical study showed that Bcl-2 expression was increased in Li-responders one month after starting Li- treatment, whereas it was decreased in Li non-responders [86]. Li has also been shown to decrease levels of pro-apoptotic proteins, including Bcl-2–associated X protein (Bax) [77, 81], and proapoptotic protein 53 (p53) [77, 80, 82, 87].

Li also decreases the activity of the Signal Transducer and Activator of Transcription 3 (STAT3), a transcription factor that has a key role in many cellular processes, including cell growth and apoptosis in response to cytokine signaling [88-92]. STAT3 activation has previously been associated with cancer proliferation and invasion and shown in various types of cancers [93, 94]. It has been suggested that Li inhibits NF-kB, a transcription factor found in most cell types that plays a key role in regulating the immune response and is found in most cell types, and reduces its pro-inflammatory and anti-apoptotic effects [95, 96]. Li reduced the activity of NF-kB in both animal and cell culture studies [97-99].

Li has been shown to activate autophagy, a crucial mechanism for the maintenance of intracellular homeostasis. Li activates autophagy through inhibition of Inositol Monophosphatase 1 (IMPase) and IP3 activation [100] in cell cultures and suppresses autophagy through inhibition of GSK-3β and activation of mTOR [101]. Li's tumor-suppressive, as well as its tumor-promoting functions, have been attributed to the above-mentioned mechanisms which regulate cell death in service of homeostasis of the living [102].

6. LITHIUM MAY INHIBIT CANCER PROLIFERATION BY MODULATING THE REDOX BALANCE: ANTIOXIDANT PROPERTIES OF LITHIUM

The outstanding body of evidence on anti-oxidant effects of Li supports the view that redox modulations may be a key mechanism in the associations of Li and cancer proliferation.

Redox imbalance occurs when the production of Reactive Oxygen Species [ROS] exceeds their elimination. It can affect cellular proteins, lipids, and nucleic acids, leading to genomic instability and activation of various signaling cascades related to carcinogenesis [103, 104]. Redox imbalance causes increases in serum/plasma levels of lipid peroxidation and protein oxidation markers [105-109] and altered serum/plasma levels of antioxidant enzymes [106, 107]. DNA/RNA damage [110-112] and impairments in DNA repair mechanisms [110, 113] exert an effect on redox status. It has been suggested that the pathophysiology of in bipolar disorder is associated with redox imbalance [114].

Evidence from preclinical studies supports that Li may contribute to redox balance and eventually decrease in DNA damage through its antioxidant properties [105, 115-119], prevention of ROS production, protein oxidation, nitration and lipid peroxidation [81, 120-124].

In clinical studies, patients with bipolar disorder have shown decreased lipid peroxidation levels after Li treatment in manic [125], depressive [126] and euthymic [127] states. Decreases in DNA fragmentation have been shown in blood cells of bipolar patients after *in vitro* incubation using mood stabilizers including Li [105, 117].

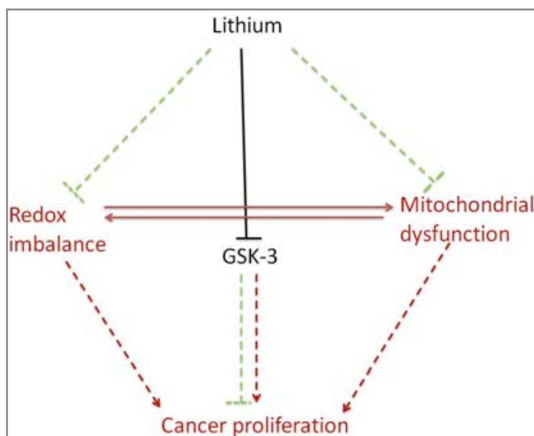

Fig. (2). The cross-talk between GSK-3 inhibition and modulations on redox balance and mitochondrial functions concerning cancer proliferation.

7. LITHIUM MAY INHIBIT CANCER PROLIFERATION BY IMPROVING MITOCHONDRIAL FUNCTIONS

Mitochondrial functioning has a significant role in the generation of ROS as by-products of respiration coupled oxidative metabolism, [114]. Impaired mitochondrial functions were linked to the neurobiology of bipolar disorder [128-130], as well as initiation and progression of many cancers [131]. Li has consistently shown protective effects on mitochondrial functions [132-134], particularly against oxidative insults [135-137]. More recently, Li has been shown to decrease cell apoptosis, and ROS production induced by mitochondrial complex I inhibitors [135-137] and increase mitochondrial complex I activity in patients with bipolar depression [138].

Excessive Ca^{2+} influx associated with increased ROS involves disruption of calcium-dependent cellular pathways, mitochondrial dysfunction, oxidative stress and activation of apoptosis [131]. Reduced Ca^{2+} signaling was found in hyperexcitable neurons of patients with bipolar disorder who responded to Li treatment [134]. Mitochondrial activities acting on closely interrelated mechanisms involving in ROS production and Ca^{2+} homeostasis represent an area for further investigations on the relationship between Li and cancer proliferation (Fig.2).

DISCUSSION

We presented the clinical case of a female patient with long-term use of Li and VPA, who developed a malignant renal tumor, which after being monitored for 10 years, was removed *via* a nephrectomy. Li was used for 18 of total 26 years of patient's mood-stabilizer treatment. The first diagnosis of a benign renal tumor was made during VPA treatment; however, the expansion of the tumor and cancer proliferation occurred during Li therapy.

The tumor was first diagnosed as angiomyolipoma, is a benign tumor which can only be assessed through ultrasonography. Although the literature describes distinctive radiologic features for angiomyolipoma, in the absence of a tissue assessment by the pathologist, it is impossible to be absolutely certain that the tumor was benign, particularly considering that the two tumor types originate from distinctively different cell lines (angiomyolipoma from blood vessels, smooth muscle cells, and fat cells; chromophobe cell carcinoma from renal tubules), and that conversion from angiomyolipoma to chromophore cell carcinoma has not been previously described [25, 27, 28].

In line with the overview of Li's effects on cancer proliferation given above, evidence shows a controversial link between Li and urinary tract cancers in particular. A different mood-stabilizer, VPA, may also have a possible role in the tumor development. VPA is known as a histone deacetylase inhibitor [139, 140]. Acetylation and deacetylation of nucleosomal core histones modulate chromatin structure and regulate gene expression. Carcinogenesis involves disruption of the balance between histone acetyltransferases (HDAC) [141]. VPA is considered among those epigenetic drugs which target chromatin through inhibiting HDACs and DNA methyltransferases [DNMTs]. VPA is a broad-spectrum inhibitor of class I and II histone deacetylases [142]. Depending on the cell type and experimental setup, VPA shows anticancer effect *in vitro* and *in vivo* through various mechanisms: induction of proliferation arrest, differentiation, and/or apoptosis, antiangiogenesis, antimetastatic, chemosensitization, radiosensitization, facilitation of immune response against tumor in a variety of human cancers such as glioma, neuroblastoma, breast, colon, prostate, thyroid, thoracic cancers, hepatoma, gynecologic cancers, and fibrosarcoma [143, 144]. Early clinical trials present encouraging results for the anticancer effect of VPA, whether used either alone or in combination with other cytotoxic agents [143, 144].

Several genes belonging to multiple pathways are shown to be upregulated in the primary human tumors treated with VPA. These include ribosomal proteins, oxidative phosphorylation, MAPK signalling; focal adhesion, cell cycle, antigen processing and presentation, proteasome, apoptosis, PI3K, Wnt signalling, calcium signalling, TGF-beta signalling, and ubiquitin-mediated proteolysis [144]. It has been shown that VPA significantly inhibits cell migration, but not the proliferation, of human kidney adenocarcinoma cell line [145]. VPA shows a dose and time-dependent induction of a significant decrease in cell proliferation and adhesion in renal carcinoma [146-148]. Based on the existing data, there is a possibility that the patient's valproate use delayed the expansion of the tumor for several years beyond its natural course.

Li's inhibition effect on GSK-3 has been the most explicated mechanism of cancer proliferation in the urinary tract, as well as in other tissues [42-44]. Aberrant nuclear accumulation of GSK-3 was found in more than 90 % of renal cell carcinoma cell lines, and a decrease in proliferation and survival of these cells was shown by inhibition of GSK-3 [149]. Moreover, GSK-3 inhibition increased the chemotherapeutic effect of sorafenib in renal carcinoma *in vitro* and *in vivo* [67], in contrast to previous suggestions that increased cell proliferation induced by Li through the potential long-term inhibition of GSK-3 inhibition leads to malignant transformation in renal cells [150, 151].

Kjaersgaard *et al.* [152] reported that rat litters exposed to Li showed urine concentrating defects and microcysts on postnatal days 7-28. This finding is in line with the suggestion that Li-induced GSK-3 inhibition could cause hyperproliferation of renal collecting duct cells and renal microcyst formation. Microcysts derive from the proliferation of epithelial cells in the distal nephron, distal convoluted and connecting tubules, as well as cortical collecting ducts [23, 152-154], and in some cases, the microcysts contain papillary projections indicative of a pre-malignant stage [22]. Previously, Markowitz *et al.* [23] reported two solid renal tumors in a series of 24 renal biopsies of patients with Li nephropathy and demonstrated that the formation of microcysts was notable, and occurred in the 62.5% of patients treated with Li. However, it is worth noting the retrospective nature of the study, and as claimed by the authors themselves, the presence of significant confounding factors could have affected the findings. First, the microcysts were also shown in patients treated with Li by several studies using renal biopsy, USG, magnetic resonance imaging [155]. Kjaergaard *et al.* [153] also reported a renal tumor in the long-term Li treated patient. Second, two reports suggested an increased risk of solid renal tumors in long-term Li users [21 22]. Rookmaaker *et al.* [22] reported 6 patients who developed solid renal tumors among 50 patients with chronic Li nephropathy over a period of 15 years. A study from France reported that the rate of renal tumors was 8.24%, and almost half of the tumors were malignant [4.12%] among 170 patients treated with Li for an average of 21.4 years, as compared to matched controls with chronic renal disease not exposed to Li [21]. The observed rate of solid renal tumors was almost six-fold greater than in patients with chronic renal disease and tenfold greater than in the general French population. However, both studies [21, 22] included patients with severely impaired renal functions. On the other hand, a meta-analysis of patients treated with Li suggested an increased risk of reduced urinary concentrating ability, but rare (almost 0.5%) progression to end-stage renal failure [156]. In the view of the results of this meta-analysis, Baldessarini and Tondo [157] commented that large samples of patients exposed to Li treatment would be required in order to effectively evaluate tumor risks among this patient group. Furthermore, Licht *et al.* [158] commented that no causal relation between Li and solid renal tumors could be inferred from the findings by Zaidan *et al.* [21] owing to methodological shortcomings (primarily selection bias). Other studies found no increased risk of renal tumors associated with Li [8, 159, 160]. In the nationwide Danish case-control study, consisting of 6477 cases and matched controls [n=259080], no increased risk of the upper urinary tract (including renal tumors) was associated with long-term Li use [159]. Another nationwide Danish cohort study included time-specific data from all individuals exposed to Li (n=24272) or anticonvulsants (n=386255), all individuals with a diagnosis of bipolar disorder (n = 9651), and a randomly selected sample from the Danish population (n=1500000) [160]. The study demonstrated no association between mood stabilizers (Li and anticonvulsants) with either malign or benign tumors of the urinary tract (renal and upper urinary tract) [160]. The period of the study was from 1995 to 2012, and the analyses were adjusted for the number of prescriptions or Li/anticonvulsants, antipsychotic agents, antidepressants, and use of all other types of medication; age; gender; employment status; calendar year; and a diagnosis of bipolar disorder [160].

Smoking is a major cause of cancer development. As patient has been a long-term smoker, in this case, it may not be possible to ignore the potential role of smoking in the tumor development. In a case series with renal tumors, 51% of the patients reported active smoking, and a history of smoking was significantly less common in chromophobe renal cell carcinoma compared to a clear cell or papillary renal cell carcinoma, indicating the distinct carcinogenic effect of smoking on different renal cell carcinoma subtypes [161]. In contrast, analysis of findings from two other case-control studies showed that smoking was similarly associated with all subtypes of renal cell carcinoma [162].

Bipolar disorder itself has been associated with increased risk for several types of cancers [163-168]. One explanation for this association may be an unhealthy lifestyle, such as obesity, tobacco smoking and alcohol use, which is more prevalent in bipolar patients than in the general population. Another explanation for cancer vulnerability in bipolar disorder, accuse the underlying neurobiology of bipolar disorder, that shows alterations in several pathways linked to cancer proliferation, including abnormal inflammatory responses, mitochondrial impairments, and redox imbalance. Supporting these two explanations, bipolar disorder has repeatedly been associated with several circumstances which are linked to cancer proliferation concurrently, including increased comorbidity [2, 3], a significant decrease in life expectancy [163, 169] and accelerated -aging [169]

In a population-based study, the cancer rate was found to be 6% in bipolar disorder patients who did not use Li, 5.9% in bipolar disorder patients who used Li and 6.4% in the general Swedish population [8]. Patients with bipolar disorder without Li treatment had a significantly higher risk for the digestive system, respiratory system and intrathoracic organs, endocrine glands and related structures cancers when compared to general population [8]. The study constituted by Huang *et al.* in Taiwan in 2016, the annual incidence of cancer in the general population was evaluated as 2.55-4.00 / 1000 [20]. The annual incidence of cancer in bipolar disorder group using Li independent of anticonvulsant treatment is 2.66 / 1000 [20].

CONCLUSION AND FUTURE REMARKS

Despite the substantial body of evidence pointing to Li's anti-carcinogenic effects in therapeutic dosages, the mechanistic pathways remain unclear on the association of Li and cancer proliferation. The limited data on the relationship between Li and cancer proliferation in clinical populations supports neither a positive relationship between long-term Li use and increased urinary tract cancers nor an overall cancer risk. However, recent clinical studies showing reduced cancer risk in bipolar patients with long-term Li treatment point to improvement in the understanding of molecular pathways involved in the link between the activity of Li and cancer proliferation. The mitochondrial dysfunction and redox modulations may be a potential area for further research on the association of Li and cancer proliferation.

LIST OF ABBREVIATIONS

HDAC	=	Histone Acetyltransferases
Li	=	Lithium
mTOR	=	Mammalian Target of Rapamycin
ROS	=	Reactive Oxygen Species
USG	=	Ultrasound
VPA	=	Valproic Acid

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

All authors contributed to reviewing literature and writing and submitting the manuscript.

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Psychiatry Research 261 (2018) 589–596

Contents lists available at ScienceDirect

Psychiatry Research

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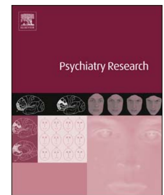

DNA redox modulations and global DNA methylation in bipolar disorder: Effects of sex, smoking and illness state

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ARTICLE INFO

Keywords:
Bipolar disorder
Redox modulation of DNA
Methylation
Sex
8-OHdG

ABSTRACT

DNA redox modulations and methylation have been associated with bipolar disorder (BD) pathophysiology. We aimed to investigate DNA redox modulation and global DNA methylation and demethylation levels in patients with BD during euthymia, mania or depression in comparison to non-psychiatric controls. The roles of sex and smoking as susceptibility factors for DNA redox modulations and global DNA methylation and demethylation were also explored. Levels of 5-methylcytosine (5-mC), 5-hydroxymethylcytosine (5-hmC) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were assessed in DNA samples of 75 patients with DSM-IV BD type I (37 euthymic, 18 manic, 20 depressive) in comparison to 60 non-psychiatric controls. Levels of 5-mC and 5-hmC were assessed using Dot Blot as a screening process, and verified using ELISA. Levels of 8-OHdG were assessed using ELISA. The levels of 8-OHdG significantly differed among non-psychiatric control, euthymia, mania and depression groups [F (3,110) = 2.771, p = 0.046], whereas there were no alterations in the levels of 5-hmC and 5-mC. Linear regression analyses revealed the significant effects of smoking (p = 0.031) and sex (p = 0.012) as well as state of illness on the levels of 8-OHdG (p = 0.025) in patients with BD. Our results suggest that levels of 8-OHdG may be affected by sex, illness states and smoking in BD.

1. Introduction

Bipolar disorder (BD) is a chronic illness leading to serious disability (Belmaker, 2004; Üstün et al., 2004). Recent evidence suggests that redox modulations (i.e. oxidative stress status) to DNA, lipids and protein are key factors involved the illness progression and severity of BD (Berk et al., 2011; Brown et al., 2014; Gigante et al., 2011; McGorry et al., 2014; Rizzo et al., 2014). Redox modulations occur when reactive oxygen and nitrogen species lead to chemical changes to lipids, proteins, and DNA (Dizdaroğlu, 2012). These are dynamic processes that can be prevented by the antioxidant system, which has been shown to be down regulated in BD (see review Andreazza, 2012).

DNA oxidative damage has been shown in both postmortem brain tissues (Buttner et al., 2007; Mustak et al., 2010; Che et al., 2010) and peripheral samples of patients with BD (Andreazza et al., 2007; Huzayyin et al., 2014; Ceylan et al., 2015; Munkholm et al., 2015; Jorgensen et al., 2013; Soeiro-de-Souza et al., 2013; Jacoby et al., 2016). Reaction between reactive oxygen species and guanosine DNA residues lead to the formation of 8-hydroxy-2′deoxyguanosine (8-OHdG), a common marker of oxidative DNA damage (Mill et al., 2008). Increased levels of 8-OHdG have been shown in serum, whole blood, and urine samples of patients with BD, as well as in patients with unipolar depression (see meta-analyses Brown et al., 2014; Black et al., 2015).

There is also a dearth of research focusing on the role of epigenetic changes in BD (Ludwig and Dwivedi, 2016). This process is characterized by modifications to gene expression through alterations in DNA methylation and chromatin structure without changing the genomic

Abbreviations: 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; BD, Bipolar Disorder; CGI, Clinical Global Impression Scale; EDTA, Ethylenediaminetetraacetic acid; ELISA, Enzyme-linked immunosorbent assay; GAF, Global Assessment of Functionality; HAM-D, Hamilton Depression Scale-17; NpC, Non-psychiatric Controls; YMRS, Young Mania Rating Scale

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https://doi.org/10.1016/j.psychres.2017.12.051
Received 3 April 2017; Received in revised form 22 November 2017; Accepted 18 December 2017
Available online 27 December 2017
0165-1781/ © 2017 Published by Elsevier Ireland Ltd.

Table 1
Demographic and clinical characteristics of study groups.

	BD (n = 75)			NpC	p-value
	Euthymia	Mania	Depression		
n	37	18	20	60	
Age (years)	36.32 ± 10.27	36.28 ± 10.31	40.80 ± 9.19	36.39 ± 11.35	F (3,132) = 0.868, p = 0.460
Body mass index (kg/m$^{2)}$	26.91 ± 4.88	29.26 ± 6.47	22.74 ± 10.29	25.94 ± 4.69	F(3,90) = 1.571, p = 0.43
Sex (female/male)	24/13	12/6	7/13	33/27	χ^2 = 5.619, df = 3, p = 0.132
Number of smokers n (%)	18 (48.6%)	7 (38.9%)	12 (60%)	17 (29.8%)	χ^2 = 6.860, df = 3, p = 0.077
Age of illness onset (years)	28.29 ± 11.49	25.59 ± 7.88	27.11 ± 7.69		F (2,69) = 1.571, p = 0.65
YMRS	0.39 ± 0.83	25.53 ± 7.83	0.16 ± 0.69		F (2,68) = 266.14, $\mathbf{p}$ **< 0.001**
HAMD-17	1.70 ± 1.63	0.47 ± 0.87	22.89 ± 4.57		F (2,68) = 449.96, $\mathbf{p}$ **< 0.001**
Number of past episodes	5.50 ± 5.52	6.78 ± 4.61	10.47 ± 5.46		F (2,70) = 5.123, $\mathbf{p}$ **= 0.008**
Presence of acute psychotic symptoms n (%)	0 (0%)	13 (72.2%)	4 (20%)		χ^2 = 36.144, df = 2, $\mathbf{p}$ **< 0.001**
Medication profiles	Drug free (n = 4) MS (n = 20) MS + AP (n = 11) AP (n = 2)	Drug free (n = 2) MS (n = 3) MS + AP (n = 13) MS + AP + AD (n = 0)	Drug free (n = 2) MS (n = 6) MS + AP (n = 4) MS + AP + AD (n = 8)		χ^2 = 27.782, df = 6, $\mathbf{p}$ **< 0.001**

Abbreviations: BD: Patients with bipolar disorder; NpC: Non-psychiatric controls; n: number; HAMD-17: 17-item Hamilton Depression Rating Scale; SSRI; YMRS; Young Mania Rating Scale; MS: receiving mood stabilizer monotherapy or combination of two mood-stabilizers; MS + AP: receiving mood-stabilizers in combination with a second-generation antipsychotic; AP: receiving antipsychotic monotherapy; MS + AP + AD: receiving mood-stabilizers in combination with an antidepressant and a second-generation antipsychotic. *statistically significant; $p < 0.05$

DNA sequence (Guo et al., 2011; Mill et al., 2008). Epigenetic changes are supported by the observations of altered methylation levels (i.e. 5mc) on promoter regions of specific candidate genes, such as Catechol-O-methyltransferase (COMT) and brain derived neurotrophic factor (BDNF), in BD (Khare et al., 2011; Nohesara et al., 2011; D'Addario et al., 2012; Dell'Osso et al., 2014). 5-Methylcytosine have important roles in cell dynamics including regulation of gene expression and maintenance of epigenetic memory, where increase in methylation levels at promoter regions will decrease gene expression (Bird, 2002). 5mc can be oxidized by the ten-eleven translocation enzyme, producing 5-hydroxymethylcytosine (5hmc), which has been associated with increased transcription of methylated DNA and regulation of the balance between methylation and demethylation (Jin et al., 2010; Wu et al., 2010).

Cytosine hydroxymethylation (i.e. 5-hmC) and guanosine oxidation (i.e. 8-OHdG) promotes DNA demethylation through decreasing the affinity of methyl groups binding to DNA CpG islands (Guo et al., 2011). Using an epigenome-wide approach, Mill et al. (2008) found epigenetic differences in genes involved in neuronal development and loci implicated in redox modulations (Mill et al., 2008). Particularly, higher 8-OHdG, lower 5-hmC levels, in the absence of significant differences in global methylation in patients with BD during manic or depressive episodes were reported (Soeiro-de-Souza et al., 2013). A possible interaction between DNA oxidation and DNA methylation has been suggested (Andreazza, 2012; Russo et al., 2016).

Several studies revealed a role of sex on redox modulations in healthy populations (Borrás et al., 2003; Loft et al., 1992; Mendoza-Núñez et al., 2010; Nakano et al., 2003; Sakano et al., 2009) as well as in various psychiatric conditions (Irie et al., 2001, 2003; Saito et al., 2013; Iida et al., 2015). Presentation and clinical course of BD show significant sex differences (Özerdem and Rasgon, 2014). However, data lacks on the effect of sex on DNA redox modulations in BD.

Several factors including age, smoking, air pollution, psychological stress, lifetime habits etc. were also associated with redox modulations (Valavanidis et al., 2009). Among these confounders, smoking has been the most replicated factor leading increased levels of 8-OHdG (Cao et al., 2016; Chen et al., 2016; Ellegaard and Poulsen, 2016; van Zeeland et al., 1999; Valavanidis et al., 2009; Yamaguchi et al., 2005).

In this study, we aimed to investigate DNA redox modulation (i.e. 8-OHdG) and global DNA methylation (i.e. 5-mc and 5-hmc) levels in patients with BD during euthymia, mania or depression in comparison to non-psychiatric controls (NpC). Secondary analysis was conducted to investigate the role of sex and smoking as susceptibility factors for DNA redox modulations and global DNA methylation. Our primary hypothesis was that patients with BD would show increased levels of DNA redox modulations and global DNA methylation compared to non-psychiatric controls. Our secondary hypothesis was that the redox modulations and methylation would vary among illness episodes. Our third hypothesis was that the altered levels of redox modulations and methylation would be affected by sex as well as smoking status beyond the illness episodes.

2. Materials and methods

2.1. Participants

Patients with BD type I and non-psychiatric controls aging between 18 and 65 years were included in the study. Seventy-five BD type I patients who met DSM-IV euthymia criteria (for at least 6 months) (n = 37), mania criteria (n = 18) or depressive episode criteria (n = 20) were recruited from the Bipolar Disorder Outpatient Unit of Dokuz Eylul University, Izmir, Turkey (First et al., 1996). DSM-IV diagnosis was confirmed with the Structured Clinical Interview for the DSM-IV Axis I Disorders and clinical variables were recorded by experienced clinicians of the research team. Symptomatic severity was assessed using Young Mania Rating Scale (YMRS) (Young et al., 1978), Hamilton Depression Scale-17 (HAM-D) (Hamilton, 1960), Clinical Global Impression Scale (CGI) (Buttner et al., 2007) and Global Assessment of Functionality (GAF) (Patterson et al., 1995).

Patients with neurological disorders, history of head trauma, chronic medical condition (e.g. hypertension, diabetes mellitus), substance use were excluded. Other exclusion criteria included comorbid Axis I psychiatric diagnosis, neurodegenerative diseases, epilepsy or previous brain surgery, auditory or visual impairment, and being pregnant or breastfeeding.

Non-psychiatric controls (n = 60) with no known medical or psychiatric history, including dementia, mental retardation, cancer, cardiovascular disease or diabetes mellitus both in themselves and in their first-degree relatives were enrolled in the study. Psychiatric conditions of the volunteers were confirmed with SCID-I interview.

Medication profiles of patients were described in four groups, drug free patients (n = 8), patients receiving mood stabilizer monotherapy or combination of two mood-stabilizers (n = 29; 9 patients on lithium monotherapy, 11 patients on valproate monotherapy, 9 patients were receiving combination of two mood-stabilizers); patients receiving mood-stabilizers in combination with a second-generation

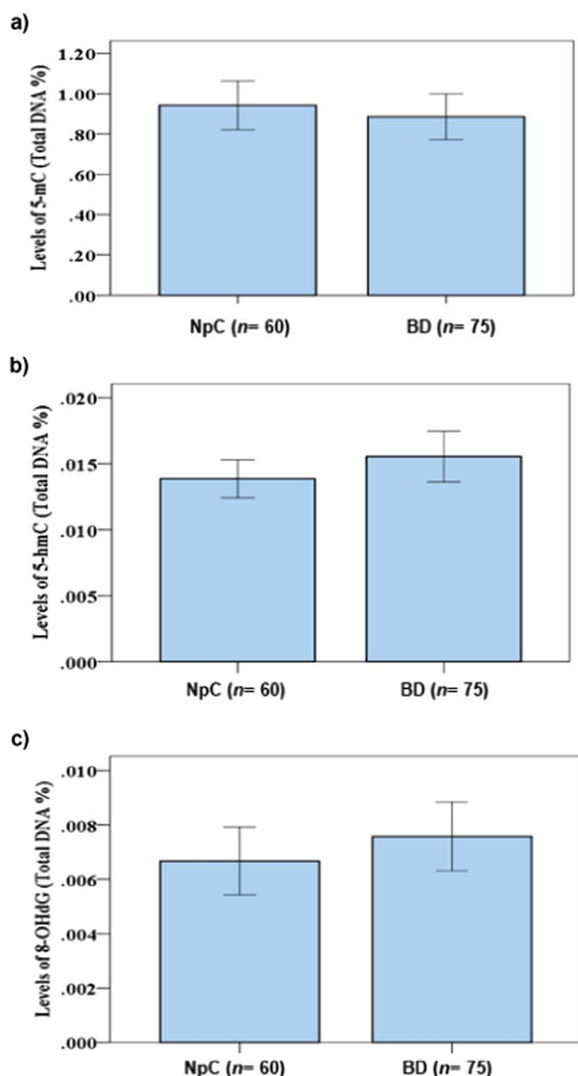

Fig. 1. DNA redox modulations and methylation in patients with bipolar disorder. Abbreviations: BD: Patients with bipolar disorder; NpC: Non-psychiatric controls; n: number; 5-hmC: 5-hydroxymethylcytosine; 5-mC: 5-methylcytosine; 8-OHdG: 8-hydroxy-2'-deoxyguanosine. Comparisons showed no significant differences between patients with bipolar disorder and non-psychiatric controls in terms of (a) 5-mC levels, (b) 5-hmC levels, (c) 8-OHdG levels.

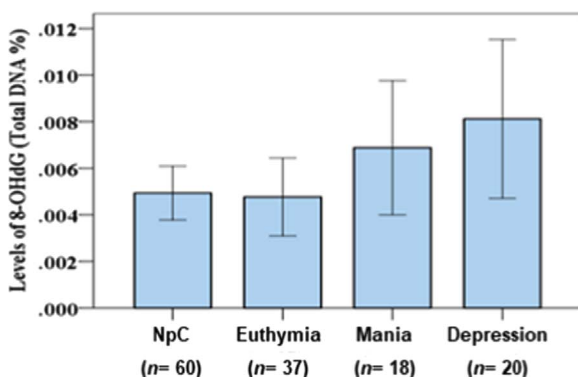

Fig. 2. The levels of 8-OHdG (total DNA %) across illness states. Abbreviations: NpC: Non-psychiatric controls; *n*: number; 8-OHdG: 8-hydroxy-2′-deoxyguanosine. The levels of 8-OHdG in patients with bipolar disorder according to the affective states ($n = 75$) compared to non-psychiatric controls ($n = 60$) were significantly different in four groups comparison [F (3,110) = 2.771, $p = 0.046$]; but there were no significant difference in post-hoc comparisons.

antipsychotic ($n = 28$); patients receiving antipsychotic monotherapy ($n = 2$), and mood-stabilizers in combination with an antidepressant and a second-generation antipsychotic ($n = 8$).

All participants provided written informed consent, and were interviewed using the Structured Clinical Interview for the DSM-IV-TR Axis I Disorders at study entry. The study was approved by Dokuz Eylul University Hospital Ethics Committee. All participants provided written informed consent.

2.2. Redox modulations of DNA and methylation assessments

Each participant provided blood in EDTA anticoagulation tubes by venipuncture between 8 and 9 a.m. DNA samples were isolated from 2 mL whole blood samples using Gen Elute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich catalogue G1N70) (Ande et al., 2015). The DNA was extracted using a silica column from Sigma and treated with RNAse. The DNA was measured spectrophotometrically and the absorbances for DNA were assessed. Ratios between these peaks were also evaluated to assess for contamination. The purity/integrity of DNA range was in between 1.8 and 2. The DNA samples were kept at −20 °C until analysis.

Levels of methylation (5-mC) and hydroxymethylation (5-hmC) were assessed using dot blot analysis as a screening process, and verified using ELISA, following previous studies (Soeiro-de-Souza et al., 2013). Absolute levels of 8-OHdG were measured using ELISA, which is a well-known method for the measurement of DNA oxidation in human biofluids (Ande et al., 2015).

2.3. Dot Blot technique

Samples were denatured in 0.4 mM NaOH and 10 mM EDTA then diluted in TE buffer (catalogue number 12090-015). Samples were boiled at 100 °C for 10 min to ensure full denaturation. Samples were spotted in duplicates onto PVDF membrane (catalogue number IPVH00010) and air dried for 1 h. The DNA was cross linked in a UV oven at 12,000 microjoules/cm^2 for 2 min. Membranes were incubated in primary antibody for 2 h, at a concentration of 1:500 (monoclonal antibodies anti-5-methylcytosine catalogue number MABE146 and anti-5-hydroxymethylcytosine catalogue number MABE176) then in 1:5000 of secondary antibody for 40 min (Gt X Rat IgG (h + L) HRP, catalogue number AP183P, for 5-hmc and Anti-mouse IgG HRP-linked, catalogue number 7076 S, for 5-mc). One hundred percent 5-mC and 5-hmC standards were used as positive controls, catalogue numbers S8005M and S8005H, respectively. Membranes were imaged using VersaDoc (Bio-Rad) on Chemi Ultra Sensitivity and 20 s of exposure time.

2.4. 5-mC and 5-hmC quantification by ELISA

Global levels of DNA methylation were quantified by measuring the amount of 5-mC in each sample. The MethylFlash™ Methylated DNA Quantification Kit (Colorimetric) provided by Epigentek was used, and the protocol was followed without major changes. One hundred ng of DNA per sample were plated for quantification. 5-hmC was measured to determine the global amount of hydroxymethylated DNA. MethylFlash™ Hydroxymethylated DNA Quantification Kit (Colorimetric) supplied by Epigentek was utilized. The protocol provided in the kit was abided by without alteration. One hundred ng of DNA were used per sample. For each protocol, a standard curve was prepared and plated as indicated in the protocol.

2.5. 8-OHdG quantification by ELISA

Absolute levels of 8-OHdG were measured using the EpiQuik™ 8-OHdG DNA Damage Quantification Direct Kit (colorimetric) from Epigentek, catalogue # P-6003. The provided protocol was followed

Table 2
DNA methylation and redox modulations levels.

BD ($n = 75$)					Group differences	
	NpC ($n = 60$)	**Euthymia ($n = 37$)**	**Mania ($n = 18$)**	**Depression ($n = 20$)**	**BD vs. NpC**[a]	**Among groups**[b]
5-hmC	0.014 ± 0.005	0.015 ± 0.007	0.018 ± 0.009	0.013 ± 0.006	0.39	F (3,127) = 0.322, p = 0.809
5-mC	0.94 ± 0.46	0.79 ± 0.50	0.90 ± 0.38	1.06 ± 0.51	0.43	F (3,123) = 1.795, p = 0.153
8-OHdG	0.0067 ± 0.0046	0.0067 ± 0.0052	0.0086 ± 0.0059	0.0087 ± 0.0038	0.26	F (3,110) = 2.771, p = **0.046**[*]

Abbreviations: BD: Patients with bipolar disorder; NpC: Non-psychiatric controls; 5-hmC: the levels of 5-hydroxymethylcytosine (total DNA %); 5-mC: the levels of 5-methylcytosine (total DNA %); 8-OHdG: the levels of 8-hydroxy-2′-deoxyguanosine (total DNA %).

[*] Statistically significant; $p < 0.05$.

[a] Independent samples t test.

[b] ANCOVA (adjusted by age, sex, smoking status.

without significant alterations. The suggested 300 ng of DNA were used for each sample. Samples were plated in duplicates. The recommended standard curve was used.

2.6. Statistical analysis

Clinical and demographic variables were compared among NpC, euthymia, mania and depression groups using independent samples *t*-tests and univariate analysis of variance (ANOVA) where appropriate. Chi-square test was used for categorical variables.

Logarithmic transformation for 5-mC and square root transformations for 5-hmC and 8-OHdG were applied to provide normal distribution in all subgroups. The normal distribution was verified using a Shapiro-Wilk test. Pearson correlation test and linear regression analyses were applied to identify the relations between clinical factors and the levels of 8-OHdG, 5-mC and 5-hmC. In line with the results of the group comparisons and correlation analyses, the illness episodes, sex, smoking status, age, presence of psychotic symptoms and medication profiles (described in 2.1) were included in the regression analyses, when analyzing patients with bipolar disorder.

A univariate analysis of variance (ANCOVA) was used to compare the levels of 8-OHdG, 5-mC and 5-hmC among study groups (i.e. NpC, euthymia, mania and depression). The variables were controlled for potential confounders including age, sex and smoking, which were associated with the levels of 8-OHdG, 5-mC or 5-hmC in linear regression analysis or previous literature on those markers (Valavanidis et al., 2009; Borrás et al., 2003). We further analyzed the non-smoker, female and male subgroups of participants. Statistical analysis was conducted using SPSS (v15.0, SPSS Inc.). The $p < 0.05$ was regarded as statistically significant.

3. Results

Sociodemographic and clinical characteristics of the sample are described in Table 1. Mean age, sex, body mass index, smoking status were similar between mania, depression, euthymia and NpC groups. However, there were significantly higher number of smokers in the entire patient group compared to the NpC group. Age of illness onset did not differ significantly among the patient groups [F (2,69) = 1.571, p = 0.65]. The number of previous episodes were significantly different among groups [F (2,70) = 5.123, p = 0.008], patients with depression had more previous episodes than euthymics (p = 0.006). Mean score of Hamilton Depression Scale and Young Mania Rating Scale were significantly different among groups [F (2,68) = 449.96, $p < 0.001$; F (2,68) = 266.14, $p < 0.001$]. All patients with BD type I had history of psychosis. Presence of acute psychotic symptoms was higher in the mania group (72.2%) than in the depressive group (20%), whereas there was no patient with acute psychotic symptoms in the euthymia group (χ^2 = 36.144, df = 2, $p < 0.001$).

3.1. DNA redox modulations and methylation in patients with BD in comparison to NpCs

Comparison of the entire BD group to the NpC group did not reveal any significant difference in terms of 8-OHdG, 5-hmC and 5-mC levels (Fig. 1). ANCOVA analyses revealed significantly different levels of 8-OHdG among four groups (NpC, euthymia, mania and depression) [F (3,110) = 2.771, p = 0.046]; whereas levels of either 5-hmC or 5-mC did not differ significantly among the same groups [F (3,127) = 0.322, p = 0.809; F (3,123) = 1.795, p = 0.153 respectively] (Fig. 2, Table 2). Despite apparently higher 8-OHdG levels in the mania and in the depression groups compared to euthymia and NpC groups, the post-hoc comparisons did not reveal any statistical significance.

3.2. Smoking effect on DNA redox modulations and demethylation/methylation

There were significantly higher number of smokers in the entire BD group when compared to NpC (Table 1). The 8-OHdG levels were significantly higher in smoker individuals in comparison to non-smoker individuals both in the entire BD group ($p < 0.017$) and in the NpC group ($p < 0.001$) (Fig. 3a). The levels of 5-mC and 5-hmC did not differ between smokers and non-smokers (p = 0.58; p = 0.29, respectively).

A significant linear regression analyses, that was conducted to identify variables that have effects on the levels of 8-OHdG [F (5,104) = 4.615; p = 0.001] with an R^2 of 0.142, revealed significant associations between smoking and higher levels of 8-OHdG in the entire study population (B = 0.019, β = 0.357, t = 4.21, $p < 0.001$, CI = 0.010–0.028), whereas no factor association between smoking status and levels of 5hmC and 5mC was identified.

To disregard the effect of smoking on the 8-OHdG levels in both patients and NpC, and to directly address the effect of illness on the DNA redox modulation and methylation we created a non-smoker subgroup of patients with BD (n = 38) and NpCs (n = 40). Comparisons in the non-smoker subgroup of participants revealed significantly different levels of 8-OHdG among NpC, euthymia, mania and depression groups [F (3,66) = 2.94, p = 0.041], and a significant interaction of sex and illness states on the levels of 8-OHdG [F (3,115) = 2.99, p = 0.034]. Despite apparent higher 8-OHdG levels in the mania group and in the depression group compared to euthymia and NpC groups, the post-hoc comparisons did not reveal statistical significance after Bonferonni corrections.

3.3. Effect of clinical variables on the redox modulations and methylation/demethylation in patients with BD

Among patients with BD, the levels of 8-OHdG, 5-mC or 5-hmC did not show any significant correlations with clinical parameters including body mass index, number of previous episodes, HAD, YMRS, GAF, CGI scores. A significant linear regression analyses, that was conducted to

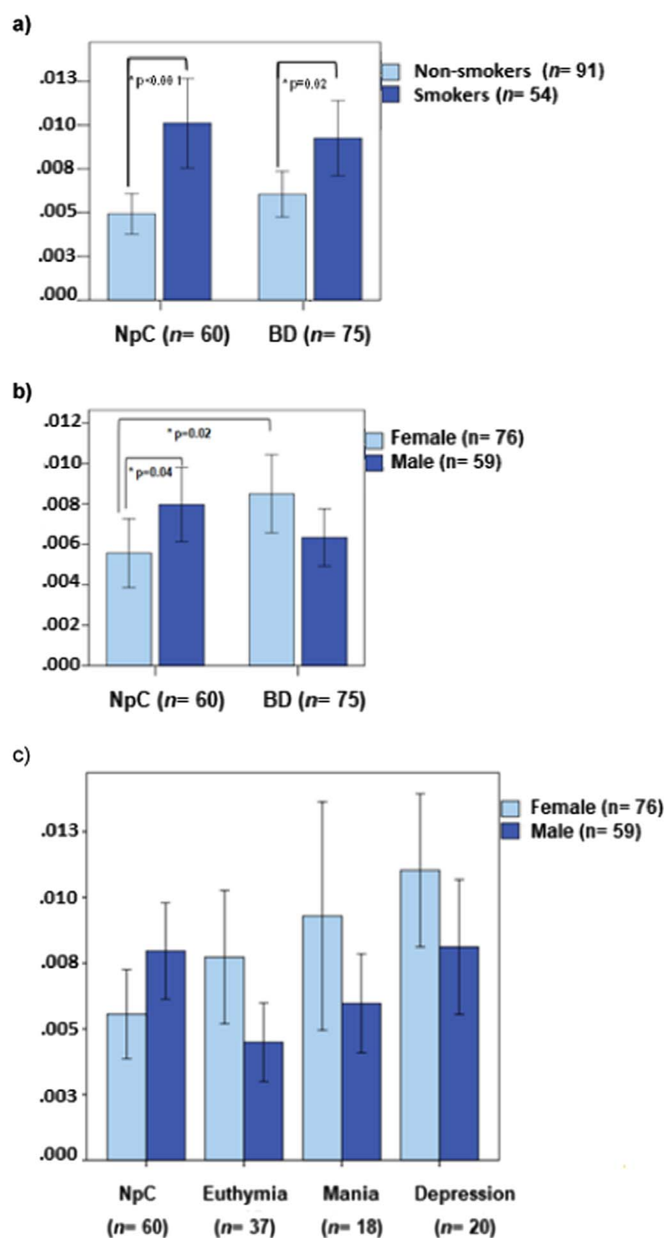

Fig. 3. The levels of 8-OHdG (total DNA %) in patients with bipolar disorder: the effects of sex and smoking status. Abbreviations: BD: Patients with bipolar disorder; NpC: Non-psychiatric controls; *n*: number; 8-OHdG: 8-hydroxy-2'-deoxyguanosine. (a) The 8-OHdG levels were significantly higher in smoker non-psychiatric controls in comparison to non-smoker non-psychiatric controls ($p < 0.001$). Within the patients with bipolar disorder, the smoker patients had higher 8-OHdG levels than non-smoker patients ($p = 0.02$). (b) Non-psychiatric males had higher levels of 8-OHdG than non-psychiatric females ($p = 0.04$). Within the female population, higher levels of 8-OHdG were detected in patients with bipolar disorder compared to non-psychiatric controls ($p = 0.02$). (c) The effect of illness episodes is more prominent in female population.

identify variables that have effects on the 8-OHdG levels of patients with BD [F(6,74) = 2.686; $p = 0.021$] with an R^2 of 0.120, revealed that state of illness (the order is euthymia, mania and depression), female sex were associated with significantly higher levels of 8-OHdG, as well as smoking ($B = 0.008$, $\beta = 0.277$, $t = 2.29$, $p = 0.025$, CI = 0.01–0.015; $B = 0.013$, $\beta = 0.249$, $t = 2.20$, $p = 0.031$, CI = 0.001–0.024; $B = 0.014$, $\beta = 0.286$, $t = 2.58$, $p = 0.012$, CI = 0.003–0.026); whereas there were no significant effects of age, treatment profiles or acute psychotic symptoms.

3.4. DNA redox modulations and demethylation/methylation in patients with BD: sex as an important susceptibility factor

Further comparisons showed that female patients with BD ($n = 43$), had significantly higher levels of 8-OHdG compared to female controls ($n = 33$) [F (2,62) = 1.69, $p = 0.02$]. Within the NpC group, females had significantly lower levels of 8-OHdG compared to males ($p = 0.04$) (Fig. 3b) as opposed to the patient group where females displayed higher levels of 8-OHdG compared to males. However, the difference did not reach statistical significance (Fig. 3b).

4. Discussion

Our results indicate significant effects of illness state, sex and smoking status on the levels of 8-OHdG in BD. Levels of 8-OHdG were still significantly different among the patient and NpC groups with a trend for increase particularly in mania and depression even after excluding smokers from the analysis showing the adverse impact of illness on the levels of 8-OHdG. Smoking was the most prominent factor associated with increased levels of 8-OHdG. The impact of the sex and illness state interaction upon 8-OHdG levels was significant in the female subgroup of the patients.

While many studies reported high levels of 8-OHdG in BD (Andreazza et al., 2008; Bengesser et al., 2014; Che et al., 2010; Siwek et al., 2013; Soeiro-de-Souza et al., 2013; Munkholm et al., 2015) or in major depressive disorder (Forlenza and Miller, 2006; Irie et al., 2002, 2003, 2005; Lindqvist et al., 2016; Maes et al., 2009; Wei et al., 2009), others found no difference in the levels of expression of this marker (Ceylan et al., 2015; Huzayyin et al., 2014; Jorgensen et al., 2013; Kupper et al., 2009). The documented discrepancy among the previous studies could be due to methodological differences in measuring DNA damage (Black et al., 2015). Recent meta-analyses, investigating the relationship between depression and oxidative stress, revealed variability in findings and connected this variability to the use of different biological specimens and laboratory techniques among the studies (Black et al., 2015). In the aforementioned studies, 8-OHdG was measured in serum by ELISA (Forlenza and Miller, 2006; Kupper et al., 2009; Soeiro-de-Souza et al., 2013; Wei et al., 2009), in urine samples by high performance chromatography (Yi et al., 2012), in urine samples by ELISA (Iida et al., 2015), in nuclear DNA from leukocytes by liquid chromatography with electrochemical detection (Kupper et al., 2009; Maes et al., 2009) or in urine samples by ultra-performance liquid chromatography and mass spectrometry (Jorgensen et al., 2013; Munkholm et al., 2015).

Our results are consistent with the previously reported serum, whole blood levels of 8-OHdG which were measured using ELISA techniques (Forlenza and Miller, 2006; Kupper et al., 2009; Soeiro-de-Souza et al., 2013; Wei et al., 2009). Consistently, a recent study with a considerably large sample size found increased urinary levels of 8-OHdG using ultra-performance liquid chromatography and mass spectrometry in all phases of rapid cycling BD (Munkholm et al., 2015). More recently, increased urinary levels of 8-OHdG in manic and depressive episodes were found using ultra-performance liquid chromatography and mass spectrometry (Jacoby et al., 2016). Notably, tissue levels of 8-OHdG reflect the balance between damage and repair rate, whereas urine excretion of 8-OHdG measures global amount of systemic oxidative stress (Poulsen et al., 2014). On the other hand, a recent study has suggested a positive relation between steady state DNA damage (plasma) and systemic DNA damage (urinary) (Wang et al., 2016).

Our finding of unchanged levels of 8-OHdG in the euthymia group compared to in the NpC is consistent with previous studies (Ceylan et al., 2015; Huzayyin et al., 2014; Jacoby et al., 2016). The increasing trend of 8-OHdG during active episodes are also consistent with previously reported elevated 8-OHdG levels in mania and depression (Andreazza et al., 2007; Soeiro-de-Souza et al., 2013; Munkholm et al.,

2015). Soeiro-de-Souza et al. (2013) had found elevated levels of 8-OHdG in a symptomatic (manic or depressive) group of patients with BD. However, despite apparently higher levels of 8-OHdG in manic and depressive states, post-hoc comparisons did not reveal significant differences between different states, possibly due to the small sample size (Type II error). On the other hand, our results revealed a significant impact of manic and depressive states on the levels of 8-OHdG. In line with our findings, in a recent study demonstrating state-related alterations of DNA damage in BD, manic and depressive groups showed similarly increased 8-OHdG levels (Jacoby et al., 2016).

Several factors, including sex and smoking affect the dynamics of DNA modulations and repair processes (Valavanidis et al., 2009; Ellegard and Poulsen, 2016; Black et al., 2016). Our results showed the significant impacts of smoking and sex as well as state of illness on the levels of 8-OHdG. In line with previous evidence, our results implicated that smoking is the most prominent factor associated with higher levels of 8-OHdG (Valavanidis et al., 2009; Black et al., 2016). On the other hand, there was a significant interaction of sex and illness episodes upon 8-OHdG levels.

Our data demonstrates significantly lower levels of 8-OHdG in healthy females compared to healthy males (Fig. 3b). Despite the lack of data focusing on the relationship between sex and DNA damage in BD, there are studies looking at healthy populations as well as unipolar depression (Borrás et al., 2003; Irie et al., 2001, 2003; Loft et al., 1992; Saito et al., 2013; Iida et al., 2015; Black et al., 2016). Previous studies in healthy populations showed higher levels of 8-OHdG in males than females (Loft et al., 1992; Black et al., 2016). In support of these findings, Borrás et al. (2003) found increased levels of mitochondrial DNA redox changes in healthy males (4-fold higher) when compared to healthy females, whereas women presented higher levels and higher activity of antioxidant enzymes, which might offer protection against redox DNA damage. In addition, Mendoza-Núñez et al. (2010) showed increased levels of glutathione in healthy females in comparison to males. Our data demonstrates significantly lower levels of 8-OHdG in healthy females when compared to healthy males, which is in concordance with the abovementioned studies.

Previous studies focusing on the impact of depressive symptoms on DNA damage reported sex differences. Negative mood, tension and ineffective coping strategies, psychological work stressors, and little prospect of stress alleviation had been related to increased DNA damage in females, whereas bereavement, adopted self-blame strategies, and perceived high workloads increased DNA damage in males (Irie et al., 2001). A population-based study reported a positive association between depressive state and 8-OHdG levels in females, oppose to males who presented a negative correlation between depressive state and 8-OHdG levels (Irie et al., 2003). Depressive symptoms were shown to be associated with higher 8-OHdG level in both young and middle-aged females (Iida et al., 2015; Hirose et al., 2016). Saito et al. (2013) reported slightly higher 8-OHdG levels among elderly female victims of natural disaster. One year after the disaster 8-OHdG levels became equal in both sexes (Saito et al., 2013). It is well known that there are significant sex differences in the presentation and clinical course of BD (Özerdem and Rasgon, 2014). Females with BD are more susceptible to bipolar type II, which may explain the relative dominance of depressive and mixed presentations in females, and rapid cycling bipolar disorder (Özerdem and Rasgon, 2014). In line with these findings, it has been suggested that male and female patients with BD may have a distinct subset of biomarkers (Chen et al., 2014).

Our results revealed significantly increased 8-OHdG levels in females with BD compared to non-psychiatric female controls and a significant interaction between sex and illness state on the levels of 8-OHdG. In concordance with the data from previous unipolar depression studies (Iida et al., 2015; Irie et al., 2002, 2003), we found that illness states were significantly associated with increased levels of 8-OHdG in female subgroup of participants. On the other hand, illness state had no effect on the levels of 8-OHdG in male subgroup of participants. Our results are in concordance with a previous population-based study, which reported a positive association between depressive state and 8-OHdG levels in females, but no association in males (Irie et al., 2003).

We investigated global methylation and hydroxymethylation. Our findings are consistent with previous reports showing unchanged global methylation levels in euthymia (Bromberg et al., 2009), and during manic and depressive episodes (Soeiro-de-Souza et al., 2013). In contrast, decreased global DNA methylation levels have been reported in euthymic patients who were excellent lithium responders (Huzayyin et al., 2014). Decreased DNA methylation levels had been shown in promoter regions of specific genes, such as Catechol-O-methyltransferase (COMT) and brain derived neurotrophic factor (BDNF) (D'Addario et al., 2012; Nohesara et al., 2011; Dell'Osso et al., 2014), suggesting that DNA methylation patterns should be extensively investigated in the promoter regions of DNA rather than global DNA.

Our data revealed no significant effect of the medication profiles on the levels of 5-hmC, 5-mC or 8-OHdG. Medications, such as mood stabilizers, antidepressants and antipsychotics that the patients received might have effects on the levels of 5-hmC, 5-mC or 8-OHdG. In a previous study, drug-free manic or depressive patients with BD presented unchanged levels of 5-mC, lower levels of 5-hmC and higher levels of 8-OHdG compared to controls (Soeiro-de-Souza et al., 2013). On the other hand, other studies investigating those parameters included medicated patients with BD (Andreazza et al., 2007; Munkholm et al., 2015; Ceylan et al., 2015; Huzayyin et al., 2014; Jacoby et al., 2016). Several studies reported that mood stabilizers, particularly lithium, might have modulating effects on redox modulations (Cui et al., 2007; Andreazza et al., 2008; Buttner et al., 2007), DNA methylation (Scola et al., 2014; Huzayyin et al., 2014; Dell'Osso et al., 2014) and hydroxymethylation (Scola et al., 2014). Further studies are needed to provide more satisfying data showing the effects of medication on redox modulations and DNA methylation/demethylation in bipolar disorder.

Certain limitations of this study including measurement technique, sample size and lack of data on potential confounders (such as hormonal status, stress status, environmental and nutritional conditions) must be highlighted. Firstly, we used the ELISA technique to measure levels of 8-OHdG and to verify the global levels of epigenetic alterations screened using Dot Blot technique. The ELISA is one of the common methods used to assess the global levels of epigenetic alterations and DNA oxidation, however, studies using some more sophisticated methods are needed to verify these results. Secondly, the absence of significant differences between different states in post-hoc comparisons, despite a significant difference among study groups revealed by covariance analysis, might be resulting from type II error due to small sample sizes of the groups. Thirdly, we do not have any data on the hormonal factors, menstrual status of the study sample, which might have contributed to dynamics of redox modulations and DNA methylation. A possible link between hormone regulation and DNA oxidation has been suggested in many studies. For instance, previous healthy population studies found that the 8-OHdG level rises in females after menopause, becoming higher than that in males, whereas the 8-OHdG levels of premenopausal females were lower than those of males (Mendoza-Núñez et al., 2010; Nakano et al., 2003; Sakano et al., 2009). Furthermore, it is suggested that a decrease in estrogen levels due to menopause inhibits the antioxidant effects of estrogen, which increases 8-OHdG levels (Sakano et al., 2009). The moderating effects of sex steroids on 8-OHdG levels, particularly those of estrogen, also need more focused research. Finally, our cross-sectional design did not allow us to adjust for environmental factors (such as diet) and personal stressors (such as workload, exercise habits). Additionally, in the absence of a stressed comparison (non-psychiatric control) group or specific measurements of the psychological stress level, our findings can not address the question whether the 8-OHdG changes are directly related to the disease process(es) or related to the stress susceptibility.

5. Conclusion

Our results suggest that levels of 8-OHdG might be affected by sex, illness states, and smoking status in BD. Our results indicate that levels of 8-OHdG display an increasing pattern in manic and depressive episodes, but not in euthymia. The impact of illness episodes (mania or depression) upon 8-OHdG levels was prominent in females. The findings highlight the importance of studying the dynamics of redox modulations across illness states in appropriate designs for controlling the potential confounders (i.e. sex and smoking). Longitudinal data is needed to further evaluate the dynamics of redox modulations across illness states within the same patients. Further studies are needed to shed a light on the dynamic of redox modulation mechanisms and the factors affecting these alterations in BD.

Acknowledgments

The authors thank the Psychiatric Association of Turkey (DC, AO; Project Research Encouragement Prize, 12.04.2011), Centre for Addiction and Mental Health Foundation (GS, LTY, MOP-484414) and Canadian Institute of Health Research (LTY, MOP-133611 and ACA, MOP-133439) as sources of funding in support of this study.

Disclosures

Dr. Ceylan and Dr. Ozerdem received research support from the Scientific and Technological Research Council of Turkey (TUBITAK). Dr. Scola has received research support from the Centre for Addiction and Mental Health. Dr. Young and Dr. Andreazza are funded by Canadian Institute of Health Research. Dr. Andreazza is funded by Ontario Mental Health Foundation and Ministry of Research and Innovation of Canada. The other authors do not have any conflicts of interest.

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DNA Repair 65 (2018) 64–72

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DNA Repair

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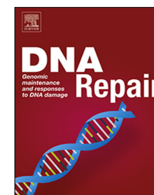

Oxidatively-induced DNA damage and base excision repair in euthymic patients with bipolar disorder

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ARTICLE INFO

Keywords:
Bipolar disorder
DNA damage
DNA repair
Base excision repair
Formamidopyrimidines

ABSTRACT

Oxidatively-induced DNA damage has previously been associated with bipolar disorder. More recently, impairments in DNA repair mechanisms have also been reported. We aimed to investigate oxidatively-induced DNA lesions and expression of DNA glycosylases involved in base excision repair in euthymic patients with bipolar disorder compared to healthy individuals. DNA base lesions including both base and nucleoside modifications were measured using gas chromatography-tandem mass spectrometry and liquid chromatography-tandem mass spectrometry with isotope-dilution in DNA samples isolated from leukocytes of euthymic patients with bipolar disorder ($n = 32$) and healthy individuals ($n = 51$). The expression of DNA repair enzymes OGG1 and NEIL1 were measured using quantitative real-time polymerase chain reaction. The levels of malondialdehyde were measured using high performance liquid chromatography. Seven DNA base lesions in DNA of leukocytes of patients and healthy individuals were identified and quantified. Three of them had significantly elevated levels in bipolar patients when compared to healthy individuals. No elevation of lipid peroxidation marker malondialdehyde was observed. The level of OGG1 expression was significantly reduced in bipolar patients compared to healthy individuals, whereas the two groups exhibited similar levels of NEIL1 expression. Our results suggest that oxidatively-induced DNA damage occurs and base excision repair capacity may be decreased in bipolar patients when compared to healthy individuals. Measurement of oxidatively-induced DNA base lesions and the expression of DNA repair enzymes may be of great importance for large scale basic research and clinical studies of bipolar disorder.

1. Introduction

Bipolar disorder (BD) is a chronic, severe and highly disabling psychiatric disorder, which is considered as one of the leading causes of disability amongst all medical and psychiatric conditions [1–3]. BD has previously been associated with increased mortality and morbidity due to general medical conditions such as cardiovascular, metabolic or inflammatory diseases [4–12]. Despite vast uncertainties about the underlying molecular mechanisms, recent evidence has shown that increased oxidatively-induced DNA damage may have a central role in the pathophysiology of BD and increased cellular aging and comorbidity in BD [13–15]. Oxygen-derived free radicals are constantly generated as

Abbreviations: BD, bipolar disorder; HI, healthy individuals; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Gua, 8-hydroxyguanine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; 5-OH-Cyt, 5-hydroxycytosine; ThyGly, thymine glycol; *S*-cdA, (5′*S*)-8,5′-cyclo-2′-deoxyadenosine; RT-PCR, real-time polymerase chain reaction; GC-MS/MS, gas chromatography/tandem mass spectrometry; LC-MS/MS, liquid chromatography/tandem mass spectrometry

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https://doi.org/10.1016/j.dnarep.2018.03.006
Received 22 February 2018; Received in revised form 29 March 2018; Accepted 29 March 2018
Available online 30 March 2018
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by-products of aerobic metabolism. Oxidative stress occurs when enzymatic and non-enzymatic antioxidant defense systems are overwhelmed by elevated levels of oxygen-derived free radicals [16]. Oxidative stress damages biological molecules such as DNA, proteins and lipids, causing multiple forms of DNA damage including base and sugar modifications, strand breaks and DNA-protein cross-links [17]. Oxidatively-induced damage to DNA can initiate mutagenic processes and early aging [18]. This type of DNA damage has been shown to play a role in the pathophysiology of cardiovascular diseases, diabetes mellitus, various cancers and psychiatric disorders including BD [18–20]. Previous studies focusing on antioxidant enzymes and oxidatively-induced damage to proteins and lipids in BD reported consistent and significant alterations in antioxidant enzymes, lipid peroxidation and nitric oxide levels [21–23]. Increased levels of DNA single- or double-strand breaks have been shown in both postmortem brain tissues [24–26] and lymphocytes of patients with BD [27]. Moreover, levels of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) have been reported to be increased in blood [28,29] and urine samples of patients with BD [30,31]. Despite a plethora of known oxidatively-induced DNA base lesions, previous research in psychiatric disorders focused on 8-OH-dG only [20]. Therefore, there are no data on the alterations of the levels of DNA base lesions other than that of 8-OH-dG in BD.

Various DNA repair mechanisms exist to repair oxidatively-induced DNA base damage. The base excision repair (BER) is the major mechanism for the repair of this type of DNA damage. It recognizes and removes modified DNA bases by DNA glycosylases, followed by the activity of other enzymes to complete DNA repair [32–34]. In BER, OGG1 is a specific enzyme for the excision of 8-OH-Gua and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), whereas 4,6-diamino-5-formamidopyrimidine (FapyAde) and FapyGua are removed by NEIL1 and NEIL3, but not 8-OH-Gua [34]. Two studies showed that increases in expression of OGG1 were associated with depressive symptoms in cancer patients [35,36]. A decrease in BER capacity in recurrent depressive disorder [37], and down regulated OGG1 levels in rapid-cycling BD [38] have also been reported.

The objective of the present study was to investigate a more extensive set of markers of oxidatively-induced DNA damage and DNA repair enzymes in DNA samples isolated from leukocytes of euthymic patients with BP in comparison to healthy individuals.

2. Materials and methods

2.1. Participants

Patients with BD ($n = 32$) and healthy individuals ($n = 51$) were included in this study. The patients who had been euthymic for at least 6 months were recruited from the Bipolar Disorders Outpatient Unit, Department of Psychiatry, Dokuz Eylul University, Izmir, Turkey. Diagnoses were confirmed using the Structured Clinical Interview for the Diagnostic Manual of Mental Disorders [39] and clinical variables were recorded by experienced clinicians of the research team. Patients with neurological disorders, history of head trauma, chronic medical condition (e.g., hypertension, diabetes mellitus) and substance use were excluded. Other exclusion criteria included comorbid Axis I psychiatric diagnosis, neurodegenerative diseases, epilepsy or previous brain surgery, auditory or visual impairment, and being pregnant or breast-feeding. Symptomatic severity was assessed using Young Mania Rating Scale (YMRS) [40], Hamilton Depression Scale-17 (HAM-D) [41], Clinical Global Impression Scale (CGI) [42] and Global Assessment of Functionality (GAF) [43]. Healthy individuals with no known medical problems, no family history of major psychiatric or no neurological disorders, including dementia, mental retardation, cancer, cardiovascular disease or diabetes mellitus in the first-degree relatives or psychiatric history were enrolled in this study. Psychiatric conditions of the healthy individuals were confirmed by the Structural Clinical Interview for DSM-IV interview [38]. The study was approved by Dokuz Eylul University Hospital Ethics Committee (Approval date: 12.07.2012; protocol no: 2012/16-13). All participants provided written informed consent.

2.2. Collection of the blood samples

Each participant provided 10 mL blood sample collected in EDTA-coagulated tubes (for leukocyte, RNA and plasma isolation) by venipuncture. At the day of the venipuncture, leukocytes were isolated from blood samples by density gradient separation using Histopaque-1119 and total RNA was extracted from 500 µL blood samples using GeneJet RNA Purification Kit (Fermentas, MA, USA). Leukocytes were frozen at −80 °C until DNA isolation. The RNA samples were frozen at −80 °C until they were converted to first-strand cDNA with an oligo-2′-deoxythymidine (dT) 18 primer. The RNA samples were converted to first-strand cDNA using the First Strand cDNA Synthesis Kit (Fermentas, MA, USA) and were frozen at −80 °C until quantitative real-time polymerase chain reaction (QRT-PCR) was performed.

2.3. DNA isolation and analysis

DNA was isolated from leukocytes by using salting-out/NaCl method [44]. DNA concentration was measured by recording the UV spectrum of each sample using an absorption spectrophotometer between the wavelengths of 200 nm and 350 nm. The absorbance at 260 nm was used to measure the DNA concentration. Subsequently, 50 µg aliquots of DNA samples were dried in a SpeedVac under vacuum. According to a Material Transfer Agreement between Dokuz Eylul University, Izmir, Turkey and National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA, DNA samples were sent to NIST for analysis by gas chromatography-tandem mass spectrometry (GC–MS/MS) and liquid chromatography-tandem mass spectrometry (LC–MS/MS).

2.4. Gas chromatography-tandem mass spectrometry

GC–MS/MS with isotope dilution was used to identify and quantify FapyAde, FapyGua, 8-OH-Gua, thymine glycol (ThyGly), 5-hydroxycytosine (5-OH-Cyt) and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd). Aliquots (50 µg) of DNA samples were supplemented with aliquots of internal standards FapyAde-$^{13}C,^{15}N_2$, FapyGua-$^{13}C,^{15}N_2$, 8-OH-Gua-$^{15}N_5$, ThyGly-$^{2}H_4$, 5-OH-Cyt-$^{13}C,^{15}N_2$ and 5-OH-5-MeHyd-$^{13}C,^{15}N_2$. DNA samples were dissolved in 50 µL of an incubation buffer consisting of 50 mM phosphate buffer (pH 7.4), 100 mM KCl, 1 mM EDTA, and 0.1 mM dithiothreitol, and then incubated with 2 µg of *E. coli* Fpg and 2 µg of *E. coli* Nth for 1 h at 37 °C to release DNA base lesions from DNA. Subsequently, 100 µL ethanol were added to precipitate DNA. After centrifugation, supernatant fractions were separated, lyophilized and trimethylsilylated. Derivatized samples were analyzed by GC–MS/MS as described previously [45].

2.5. Liquid chromatography-tandem mass spectrometry

LC–MS/MS with isotope dilution was used to measure the levels of (5′*S*)-8,5′-cyclo-2′-deoxyadenosine (*S*-cdA) and 8-OH-dG, which is the 2′-deoxynucleoside form of 8-OH-Gua. Aliquots of *S*-cdA-$^{15}N_5$ and 8-OH-dG-$^{15}N_5$ as internal standards were added to an aliquot of 50 µg of DNA samples, which were then dried in a SpeedVac. Subsequently, DNA samples were hydrolyzed with a mixture of nuclease P1, phosphodiesterase I and alkaline phosphatase according to a published procedure [45]. All samples were filtered using Millipore Microcon Ultracel YM-3 ultrafiltration membranes (Millipore, Bedford, MA) with molecular mass cutoff of 3 kDa by centrifugation at 12000xg for 30 min. LC–MS/MS analyses were performed using a Thermo-Scientific Finnigan TSQ Quantum Ultra AM triple quadrupole MS/MS system with an installed heated electrospray-ionization source, as described previously

Table 1
Demographic and clinical characteristics of the participants. Uncertaities are standard deviations.

	BD patients (*n* = 32)	Healthy individuals (*n* = 51)	*p*-value
Gender (number of females; their percentage)[a]	20; 62.5%	30; 58.8%	0.74
Age[b]	37.63 ± 9.96	36.28 ± 11.45	0.58
Smoking status (number of smokers; their percentage)[a]	15; 46.9%	16; 31.4%	0.16
Age of onset of illness[b]	26.83 ± 10.21		
Duration of illness (years)[b]	10.63 ± 8.72		
Total number of episodes[b]	5.63 ± 4.34		
Number of manic episodes[b]	3.20 ± 2.59		
Number of depressive episodes[b]	2.20 ± 2.46		
Clinical Global Impressions scale[b]	1.23 ± 0.43		
Global assessment of functionality[b]	86 ± 6.21		
Hamilton Depression Scale[b]	1.17 ± 1.49		
Young Mani Rating Scale[b]	0.47 ± 0.97		

[a] Chi-Square.
[b] independent samplest test.

[46]. Hydrolyzed DNA samples (20 µL injection volume, no waste mode) were analyzed using a Zorbax SB-Aq rapid resolution narrow-bore LC column (2.1 mm x 150 mm, 3.5 µm particle size) (Agilent Technologies, Wilmington, DE) with an attached Agilent Eclipse XDB-C8 guard column (2.1 mm × 12.5 mm, 5 µm particle size). In all instances, the autosampler and column temperature were kept at 5 °C and 40 °C, respectively. Mobile phases A and B were water and acetonitrile, respectively, both containing 0.1% formic acid (v/v). A gradient analysis of 3% (v/v) of B/min starting from 98% A/2% B (v/v) was used. After 6 min, B was increased to 60% in 0.1 min and kept at this level for 1 min and then another 13 min at 2% to equilibrate the column. The flow rate was 0.5 mL/min and the total analysis time was 20 min. Analysis by LC–MS/MS was performed using selected-reaction monitoring mode with the mass transitions *m/z* 284 → *m/z* 168 and *m/z* 289 → *m/z* 173 for 8-OH-dG and 8-OH-dG-$^{15}N_5$, respectively, and with the mass transitions *m/z* 250 → *m/z* 164 and *m/z* 255 → *m/z* 169 for *S*-cdA and *S*-cdA-$^{15}N_5$ respectively.

2.6. Measurement of the expression levels of DNA repair enzymes

The mRNA expression levels of human OGG1 and human NEIL1 were determined in samples of BD patients (n = 17) and healthy individuals (n = 19). Expressions of NEIL1 and OGG1 were measured by QRT-PCR using Maxima Sybr Green qPCR Master Mix (2x) (Fermentas, MA, USA) Kit. β-Actin was used as housekeeping gene. The amplification was performed in Light Cycler 1.5 (Roche Applied Science, Penzberg, Germany). Three independently prepared samples were used for each data point. The difference of cycle of threshold (Ct) between reference and target gene locus was observed by normalizing using housekeeping gene and calculating ΔΔCt ratio (ΔΔCt = ΔCt sample − ΔCt reference). Gene expression levels were calculated using the formula $2^{-\Delta\Delta Ct}$ [47,48].

2.7. Measurement of malondialdehyde

Malondialdehyde was extracted and analyzed according to a previously described method with slight modifications [49]. Briefly 40 µL plasma was diluted with 100 µL of H_2O and mixed with 20 µL of 2.8 mmol/L BHT in 95% ethanol, 40 µL of 81 g/L sodium dodecyl sulfate, and 600 µL of thiobarbituric acid (TBA) reagent consisting of 8 g/L TBA diluted 1:1 with 200 mL/L acetic acid adjusted to pH 3.5 with NaOH. The mixture was immediately incubated in a 90 °C water bath for 60 min and cooled on ice; 200 µL of H_2O and 1 mL of butanol-pyridine (15:1 by vol.) were then added. After vigorous mixing, the organic layer was separated by centrifugation (10 min at 10000 rpm). An aliquot (10 µL) was directly injected onto the high-performance liquid chromatography (HPLC). Calibration curves were constructed using 1,1,3,3-tetraethoxypropane (0.75 µmol/L–40 µmol/L). The separation of the extracts was performed on an automated Shimadzu HPLC system (VP Series, Kyoto, Japan). The analytical column was a reverse phase silica based C18 column (GL Sciences/Inertsil ODS-3), with column dimensions of 150 × 4.6 mm, 5 µm. The mobile phase consisted of 70% 10 mM KH_2PO_4, pH 7.0 and 30% MeOH. The sample run was 5 min, with a flow rate of 0.8 mL/min, and fluorescence detection at 515 nm (excitation) and 553 nm (emission).

2.8. Statistical analyses

The IBM SPSS Statistics 23.0 (Chicago IL, USA) for Windows was used for data analysis. The Shapiro-Wilk's test was used to confirm normal distribution for continues variables. Where necessary, logarithmic transformations were applied in order to improve normality. Subsequently, transformed data were reassessed for normality. Group differences on continuous variables regarding demographic and clinical variables were evaluated with independent samples *t*-test. Chi-Square test was used to examine categorical data.

The statistical analysis of the GC–MS/MS and LC–MS/MS data was performed using the GraphPad Prism 7.01 software (La Jolla, CA, USA) and the unpaired, two-tailed nonparametric Mann Whitney test with Gaussian approximation and confidence level of 95%–99%. A *p*-value < 0.05 was assumed to correspond to statistically significant difference between medians.

3. Results and discussion

Sociodemographic and clinical characteristics of the BD patients and healthy individuals are described in Table 1. The groups did not differ from each other with regard to gender, age or smoking. One of the patients was drug-free, 9 patients were on mood-stabilizers as monotherapy (lithium or valproate), 19 patients were receiving a mood-stabilizer in combination with a second generation-antipsychotic, and one patient was receiving a mood-stabilizer in combination with an antidepressant. We identified and quantified six DNA base lesions by GC–MS/MS and two modified 2′-deoxynucleosides by LC–MS/MS in DNA samples from both BD patients and healthy individuals. The structures of these lesions are given in Fig. 1. It should be noted that 8-OH-dG is the 2′-deoxynucleoside form of 8-OH-Gua. Fig. 2A–H show the levels of the lesions shown in Fig. 1. A large group of samples was used for the measurements by GC–MS/MS. *S*-cdA and 8-OH-dG were measured in the remaining samples by LC–MS/MS. In various samples, some lesions could not be quantified with certainty. Therefore, the number of patient samples and that of healthy individual samples in the figures somewhat differ from lesion to lesion. The number of samples in each case is given in the legends of the figures. The levels of FapyAde, FapyGua and 5-OH-5-MeHyd in BD patients were significantly greater than those in healthy individuals. The confidence level was even 99%

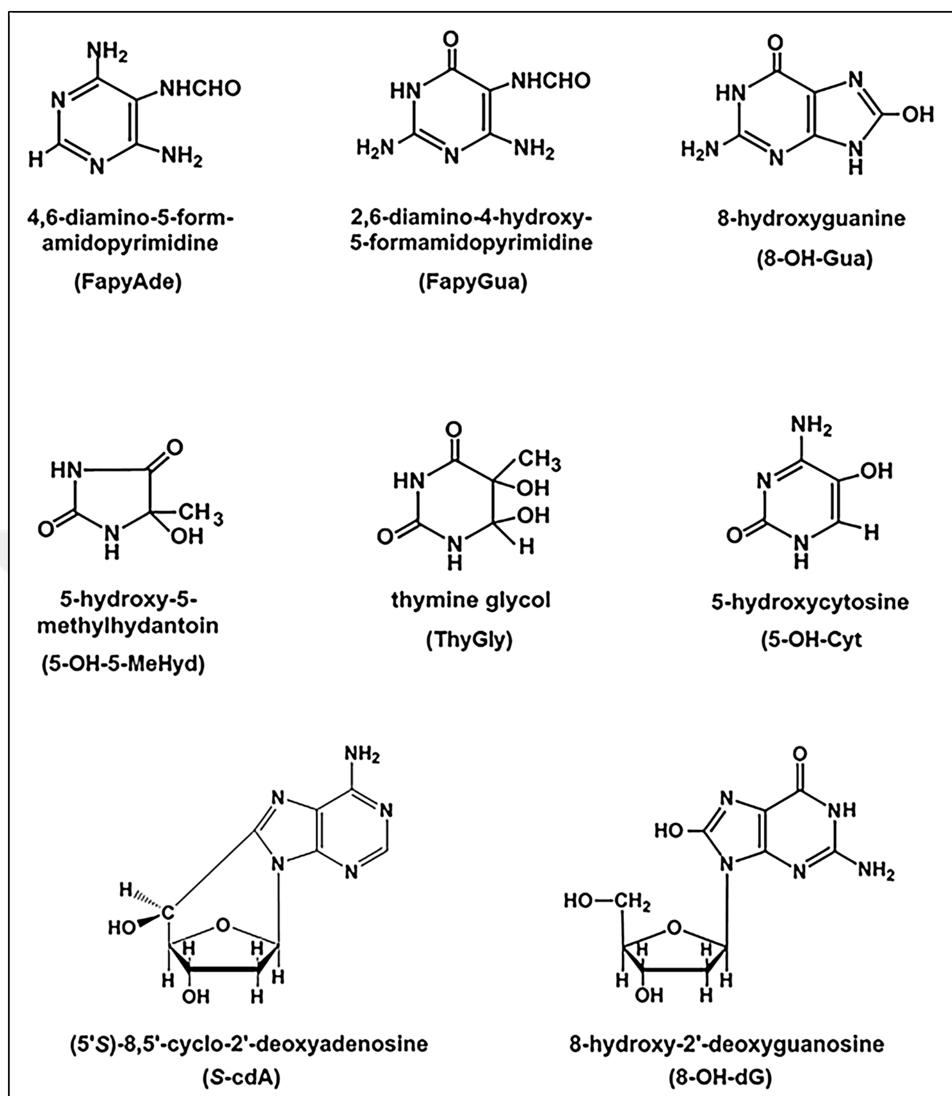

Fig. 1. The structures of the DNA lesions measured in this work.

for the latter two lesions. No significant difference between the levels of other lesions was observed when both groups were compared. Similarly, patient and healthy individual groups did not differ significantly with regard to the levels of malondialdehyde (Fig. 3). Fig. 4 illustrates the expression levels of OGG1 and NEIL1. The expression level of OGG1 in BD patients was 43% lower than that in healthy individuals and the difference between the two groups was significant after adjusting for age, gender and smoking (F = 3.278, df = 4, p = 0.007). On the other hand, no significant difference was observed between the expression levels of NEIL1 in both groups (p = 0.49) (Fig. 4).

Our results show that oxidatively-induced DNA damage occurs in DNA of BD patients compared to healthy individuals. Three out of the 8 DNA lesions measured in the present work exhibited significantly greater levels in BD patients than those in healthy individuals. To the best of our knowledge, this is the first study assessing different types of DNA lesions, representing oxidatively-induced damage to all four DNA bases in BD patients. The current knowledge on DNA base damage in BD has been based on the levels of 8-OH-dG only, which has been mostly measured as "*the most prominent DNA base lesion*" in biological samples because of the limitations of the methodologies used. Therefore, there were no available data on the lesions derived from adenine, cytosine and thymine, and one other important lesion of guanine, i.e., FapyGua. To this end, it is well known that hydroxyl radical attack on Gua produces both 8-OH-Gua and FapyGua by oxidation and reduction of the same Gua–OH-adduct radical, respectively. Moreover, the yields of these products depend on the reaction conditions [50].

In the present work, 8-OH-Gua and 8-OH-dG were measured by two different techniques. It is important to note that GC–MS/MS and LC–MS/MS yielded almost identical levels of these compounds. In both cases, the levels 8-OH-Gua and 8-OH-dG did not differ between BD patients and healthy individuals. In contrast, the level of FapyGua was found to be significantly greater in BD patients than in healthy individuals (p = 0.006 with the confidence level of 99%). Thus, our results clearly show that the measurement of one DNA lesion such as 8-OH-dG (or 8-OH-Gua) only does not necessarily prove whether DNA damage in a given biological system occurs or not. Past published data on 8-OH-dG in BD patients differed among the studies. For example, a meta-analysis of the existing data [51] and more recent studies [29,30] showed greater levels of 8-OH-dG in BD patients than in healthy individuals, whereas several other studies reported unchanged levels of 8-OH-dG in both cases [28,30,52]. The discrepancy among these findings may be due to the methodological differences between the studies, and to the differences between clinical features of the study populations including illness state, course of illness, medications, smoking status, etc.

We also measured the expression levels of OGG1 and NEIL1 in both BD patients and healthy individuals. The expression level of OGG1 was

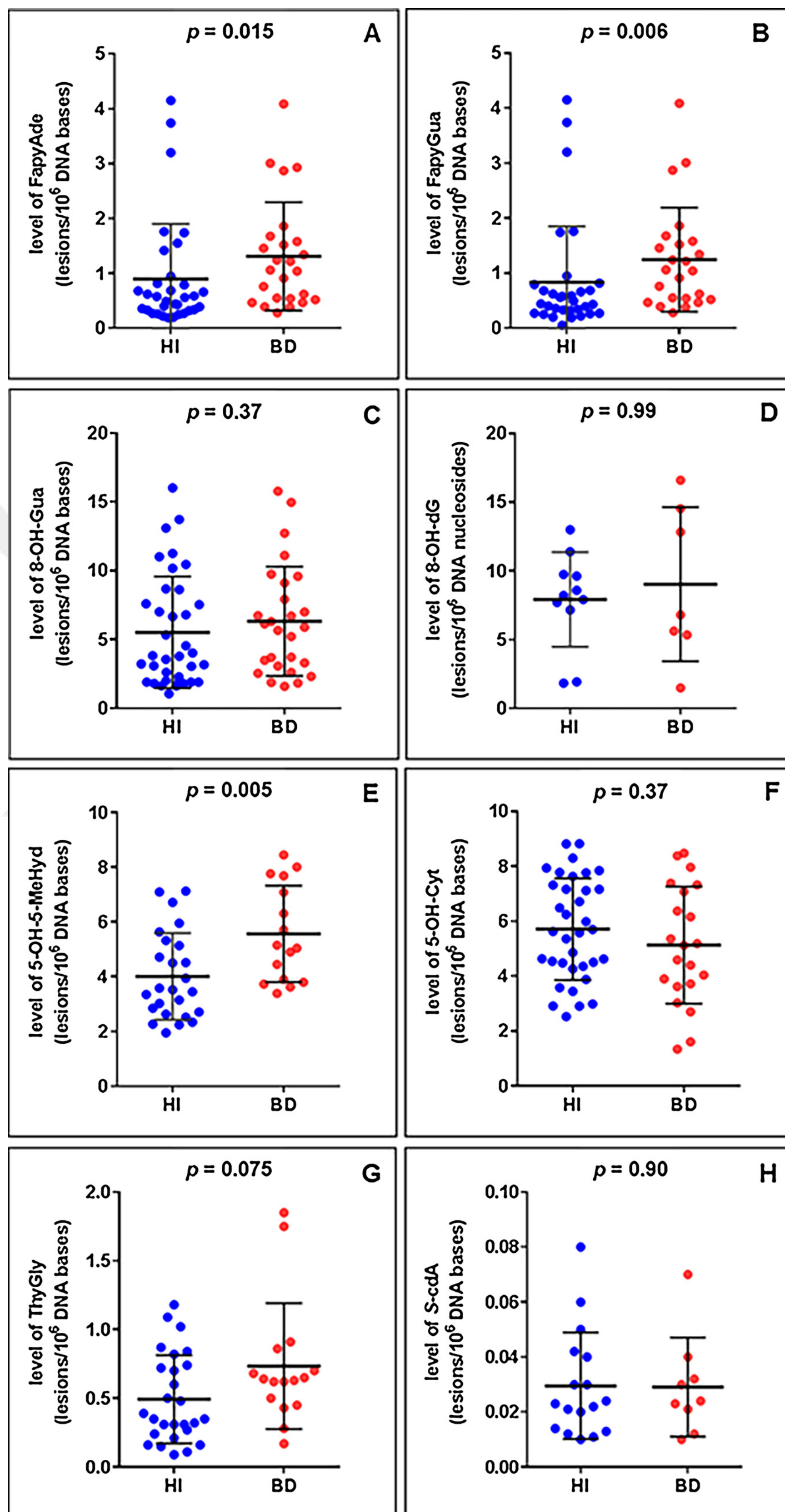

Fig. 2. The levels of DNA lesions. A: FapyAde in healthy individuals (n = 33) and BD patients (n = 25); B: FapyGua in healthy individuals (n = 32) and BD patients (n = 24); C: 8-OH-Gua in healthy individuals (n = 36) and BD patients (n = 27); D: 8-OH-dG in healthy individuals (n = 11) and BD patients (n = 7); E: 5-OH-5-MeHyd in healthy individuals (n = 25) and BD patients (n = 16); F: 5-OH-Cyt in healthy individuals (n = 35) and BD patients (n = 21); G: ThyGly in healthy individuals (n = 28) and BD patients (n = 16); H: *S*-cdA in healthy individuals (n = 17) and BD patients (n = 9). Uncertainties are standard deviations.

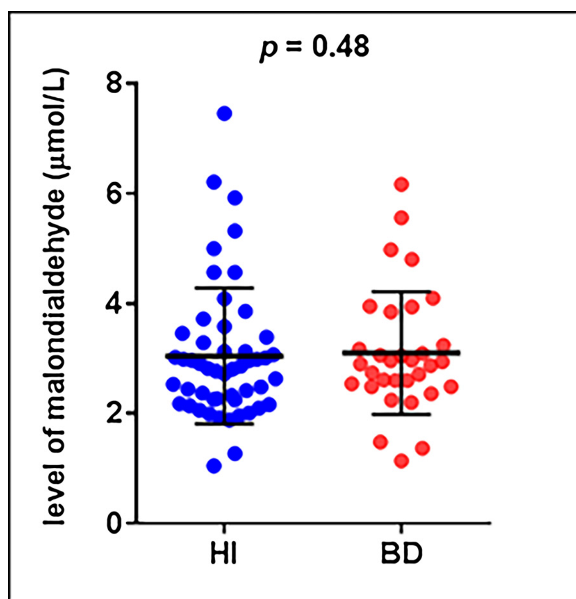

Fig. 3. The level of malondialdehyde in healthy individuals ($n = 51$) and BD patients ($n = 31$). Uncertainties are standard deviations.

found to be lower in BD patients than in healthy individuals. This is on a par with the previously reported down-regulated OGG1 expression in a rapid-cycling group of BD patients [38]. On the other hand, no elevation of NEIL1 expression was observed in BD patients. OGG1 and NEIL1 are bifunctional DNA glycosylases that are involved in the first step of the BER pathway to remove modified DNA bases from damaged DNA [32–34]. Their specificities differ from each other in that OGG1 removes FapyGua and 8-OH-Gua with similar excision kinetics, whereas NEIL1 is mainly specific for FapyAde and FapyGua, and to a lesser extent for 5-OH-5-MeHyd and ThyGly, but not for 8-OH-Gua [34]. In general, DNA glycosylases possess broad specificities for removal of DNA base lesions. For example, besides NEIL1, NTH1 and NEIL3 are specific for FapyAde removal in mammalian cells. The latter also removes FapyGua. NTH1 is the major DNA glycosylase that acts on ThyGly and 5-OH-Cyt in mammalian cells [33,34]. Therefore, the correlation of the levels of DNA base lesions with the expression levels of DNA glycosylases is quite complex, and not well understood. Low expression level of OGG1 in BD patients may be one of the factors leading to the greater level of FapyGua. On the other hand, similar levels of NEIL1 in both groups did not seem to affect the significant accumulation of FapyAde and 5-OH-5-MeHyd in BD patients. To this end, it is well known that various polymorphic variants of NEIL1 exist in human population such as NEIL1-Ser82Cys, NEIL1-Gly83Asp, NEIL1-Cys136Arg, NEIL1-Asp252Asn and NEIL1-Pro208Ser (reviewed in [33,34]). Among these variants, NEIL1-Gly83Asp and NEIL1-Cys136Arg have been shown to be completely devoid of glycosylase activity. Furthermore, NEIL1-Gly83Asp and NEIL1-Cys136Arg had significantly reduced activity. Such polymorphic variants may affect their binding, catalytic activity or protein–protein interaction with other DNA repair proteins such as PARP1, XRCC1 and CSB. These effects may cause the accumulation of typical substrates of NEIL1 such as FapyAde, FapyGua and 5-OH-5-MeHyd, as was found in this work. Future studies might include exomic sequencing of the NEIL1 gene to examine for such polymorphisms in BD patients.

The increased levels of oxidatively induced DNA lesions observed might be explained by increased oxidative stress in BD. Previous studies reported several alterations in oxidative markers including lipid peroxidation markers, antioxidant enzymes and nitric oxide levels in BD [53,54]. Malondialdehyde has been one of the most consistent lipid peroxidation marker that was found to be elevated in BD [55]. However, our results demonstrated no significant alterations in malondialdehyde levels, implying unchanged levels of oxidative stress load in the patient population compared to healthy individuals.

Several studies suggest that BD is associated with increased incidence for several medical comorbidities including cardiovascular, endocrine, inflammatory diseases [4–12], as well as various types of cancers [56–59]. DNA damage and reduced DNA repair capacity have been suggested to be one of the key mechanisms that underlie high clinical comorbidity, vulnerability to several cancers, neurocognitive decline and early aging in BD patients [13–15]. Some of the DNA base lesions identified in this work are strongly mutagenic and thus may contribute to those symptoms and others in BD patients. Thus, 8-OH-Gua and FapyGua pair with non-cognate Ade and lead to G → T transversion mutations [60–64]. The level of 8-OH-Gua was not increased in BD patients; however, 8-OH-Gua is readily oxidized, leading to the formation of spiroiminohydantoin (Sp) and 5-guanidinohydantoin (Gh), which exhibit mutagenic effects as well as cytotoxic effects [65]. Sp and Gh were not measured in the present work. Facile oxidation of 8-OH-Gua may prevent its accurate measurement *in vivo*. FapyAde leads to A → T transversions and is mutagenic, albeit to a lesser extent than FapyGua [66]. 5-OH-5-MeHyd can be a lethal or mutagenic lesion, because it constitutes a replication block for some DNA polymerases or is by-passed by low fidelity polymerases [67–70] (for more information on the mutagenic effects of oxidatively-induced DNA base lesions identified in this work, see reviews [33] and [71]).

Clinical characteristics of the patient population of this study needs consideration while interpreting our results. It is important to note that our study population consisted of only euthymic patients with BD. Previously, manic or depressive patients were shown to have higher levels of 8-OH-dG lesions than euthymic patients [28,31]. Further studies are needed to identify different types of DNA lesions presenting oxidatively-induced damage to all DNA bases measured in this study and DNA repair enzyme profiles across different states of BD (mania, depression and euthymia). Smoking status of participants might affect the levels of DNA damage/repair [72,73]. In this work, however, double comparisons between smokers and non-smokers did not show any significant difference with respect to DNA lesions and DNA glycosylases (Table 2).

Medication effect is the other parameter that requires attention while studying DNA damage in any patient population. Our patient population was predominantly on mood stabilizing medications (i.e., lithium or valproate). Previous evidence suggested that lithium and valproate treatments may have antioxidant properties [74–79], and may decrease DNA damage [24,74–79]. However, some studies showed similarly higher 8-OH-dG levels in both unmedicated [29] and medicated patient populations [27,28,30,31], leaving the effect of psychotropic medications on DNA damage equivocal. Future prospective studies specifically designed to understand the effects of mood stabilizing treatments on DNA damage/repair processes are needed.

In conclusion, our results show enhanced levels of several oxidatively-induced DNA base lesions and reduced levels of OGG1 in leukocytes of patients with BD when compared with healthy individuals. These findings suggest a defect in base excision repair in BD. Measurement of oxidatively-induced DNA base lesions and expression levels of DNA repair enzymes may be of great importance for large scale basic research and clinical studies of BD, contributing to a comprehensive understanding of the DNA damage/repair mechanisms in BD.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

DNA samples were transferred to NIST from Dokuz Eylul University and analyzed at NIST pursuant to a Material Transfer Agreement

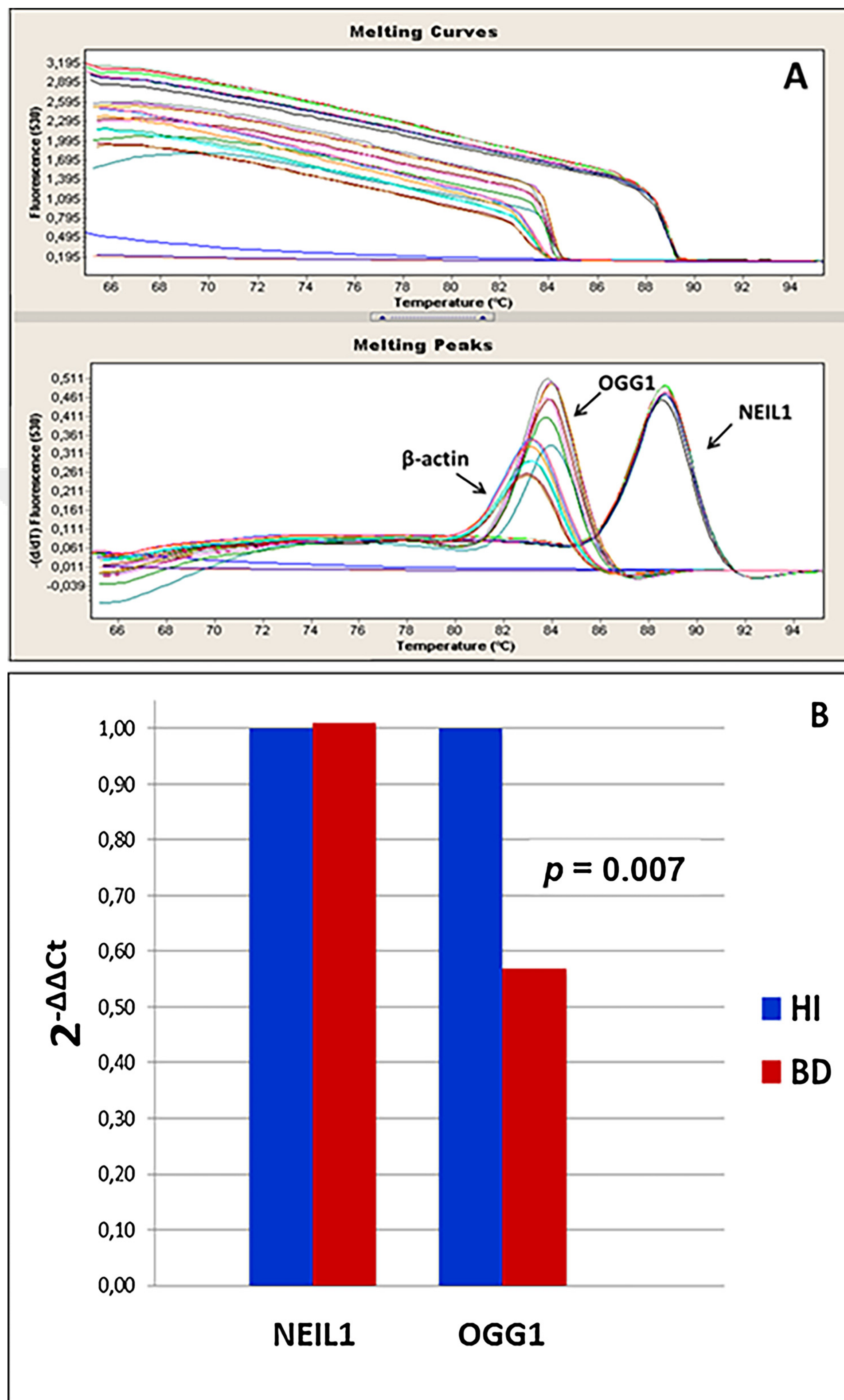

Fig. 4. A: Analysis of the melting curves of the OGG1, NEIL1 and β-actin (housekeeping) genes: The coefficient of variations (% CV) for the expressions of the genes NEIL1, OGG1 and β-actin were 0.6, 1.2 and 1.5, respectively. B: Expression profiles of OGG1 and NEIL1: OGG1 ($2^{-\Delta\Delta Ct}$) 0.43 (↓) (p = 0.007) and NEIL1($2^{-\Delta\Delta Ct}$) 0.01 (↑) (p = 0.489). The results are adjusted by age, gender, smoking status using lineer regression. $2^{-\Delta\Delta Ct}$ (fold change) is computed using α = 0.016. Ct: cycle of threshold; ΔCt: the difference of Ct between target gene and β-actin (housekeeping) gene.

Table 2
Comparisons of markers between smokers and non-smokers. Uncertainties are standard deviations.

	Non-smoker participants (n = 31)	Smoker participants (n = 52)	p-value[a]
DNA lesion (lesions/10^6 DNA bases)			
FapyAde	1.06 ± 1.11	1.35 ± 1.26	0.249
FapyGua	0.90 ± 0.80	1.22 ± 1.10	0.234
8-OH-Gua	6.30 ± 4.44	5.15 ± 3.12	0.484
8-OH-dG	8.38 ± 3.77	8.32 ± 5.59	1.000
5-OH-5-MeHyd	4.38 ± 1.75	5.27 ± 1.94	0.140
5-OH−Cyt	5.25 ± 2.03	5.85 ± 1.84	0.360
ThyGly	0.57 ± 0.39	0.67 ± 0.47	0.788
S-cdA	0.03 ± 0.02	0.03 ± 0.02	0.357
Malondialdehyde (μmol/L)	3.06 ± 1.25	3.08 ± 1.11	0.843
DNA glycosylases			
OGG1 (ΔCt)	6.31 ± 0.83	6.46 ± 0.89	0.566
NEIL1 (ΔCt)	2.76 ± 0.47	2.77 ± 0.51	0.986

Ct: cycle of threshold; ΔCt: the difference of Ct between target gene and β-actin (housekeeping) gene.
[a] Mann-Whitney U test.

between NIST and Dokuz Eylul University. Certain commercial equipment or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. The Psychiatric Association of Turkey awarded an Encouragement Prize to this research project.

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