UNIVERSIDADE FEDERAL DE MINAS GERAIS

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VERIFICAÇÃO DE COMPRIMENTO TELOMÉRICO EM PACIENTES COM TRANSTORNO AFETIVO BIPOLAR E SUA RELAÇÃO COM ASPECTOS CLÍNICOS

Belo Horizonte, MG

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Dissertação apresentada como requisito à obtenção do título de Mestre junto ao Programa de Pós-Graduação em Medicina Molecular da Universidade Federal de Minas Gerais

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RESUMO

Introdução: O transtorno afetivo bipolar (TAB) é caracterizado por alterações do humor e progressiva piora nos prejuízos funcionais. Telômeros são estruturas complexas que se localizam à extremidade dos cromossomos e os protegem de recombinação e degradação cromossômica. O encurtamento de telômeros está associado ao envelhecimento celular, já que acontece à medida em que ocorrem divisões celulares, e pode ser acelerado em exposição ao estresse. **Objetivo:** Caracterizar a ocorrência deste encurtamento, assim como verificar fatores de risco para o mesmo. **Método:** Foram convidados 85 pacientes com TAB e 95 sujeitos controle pareados por idade, sexo e escolaridade. Ambos os grupos passaram por entrevistas psiquiátricas e avaliações clínicas. O material genético, foi submetido ao PCR quantitativo em tempo real para verificação do comprimento de telômeros. **Resultados:** Pacientes apresentaram telômeros mais curtos quando comparados aos controles, apesar de não apresentarem nenhum fator de risco específico para tal encurtamento. O encurtamento telomérico não ocorreu de forma homogênea ao longo do grupo de pacientes. Não houve diferenças quando comparados pacientes e controles de telômeros longos, este resultado aponta para presença de fatores de proteção neste subgrupo clínico de pacientes. **Conclusões:** É possível que o encurtamento telomérico acelerado apresente-se como marcador de vulnerabilidade no transtorno bipolar.

Palavras-chave: Transtorno Afetivo Bipolar, Telômeros, Saúde Mental.

ABSTRACT

Introduction: Bipolar Disorder (BD) is a mood disorder with progressive worsening in functional impairment. Telomeres are a nucleoproteic complex at chomossomes ends that protect them from end to end fusion and DNA degradation. Telomeres shorten as cell division occurs and it is associated with celular aging and can be accelerated as a result of stress exposure. **Objective:** Characterize bipolar telomere shortening dynamics, as well as to verify its risk factors in BD sample. **Metlhod:** Eighty five BD patients and 95 healthy controls were paired for age, sex and educational level. Volunteers were submitted to a psychiatric interview and clinical evaluation. It was performed a Real Time Quantitative PCR in order to verify telomere length. **Results:** Patients presented shorter telomeres when compared to controls, athough no specific clinical features was stablished as a risk factor. Telomere shortening did not occur homogeneously along BD group, since no significant differences were found between patients and controls belonging to long telomere category. **Conclusions:** This result points to the presence of protective factors within this subgroup of BD patients. It can be concluded that accelerated telomere shortening is a vulnerability marker, which is associated with stress exposure.

Key-words: Bipolar Disorder, Telomeres, Mental Health.

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LISTA DE ABREVIATURAS E SIGLAS

ATM: Ataxia telangiesctasia mutated

Bp: Basepairs

CMV: Citomegalovírus

Ct: Threshold Cycle

DNA: Deoxyribonucleic Acid

Kb: Kilobase

TERT: Telomerase Reverse Transcriptase

TERC: Telomerase RNA component

HPA: Hypothalamic: pituitary adrenal axis

IL: Interleukin

NK: Natural Killer Cells

PBMCs: Células Mononucleares Sanguíneas Periféricas

PCR: Polymerase Chain Reaction

RNA: Ribonucleic Acid

SCZ: Schizophrenia

SRO: Espécies Reativas ao Oxigênio

TAB: Transtorno Afetivo Bipolar / BD: Bipolar Disorder

TBARS: Thiobarbituric Acid Reactive Substances

TC: Telomere Content

TNF: Tumor Necrose Factor

UV: Irradiação Ultravioleta

SUMÁRIO

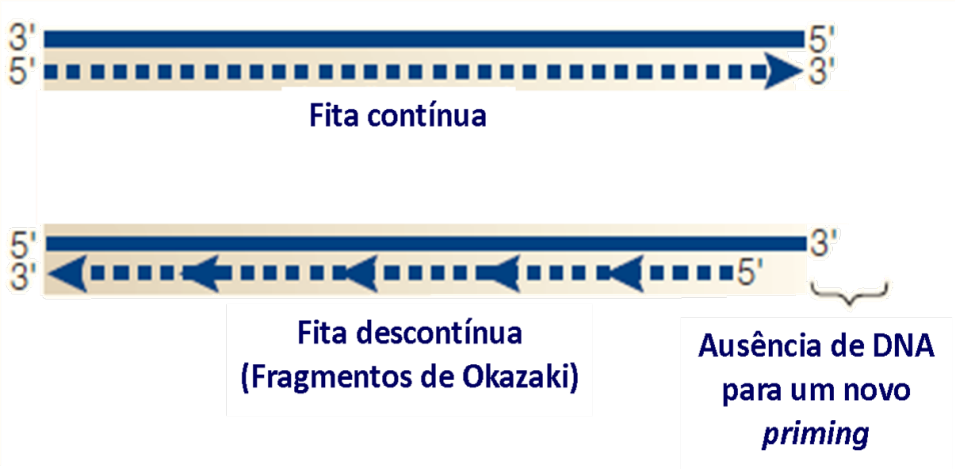
1. INTRODUÇÃO

1.1. TELÔMEROS, "O PROBLEMA DO FINAL REPLICAÇÃO"

Nos anos de 1960, Hayflick descreveu o processo de senescência em culturas celulares de tecido humano. O processo de crescimento das culturas foi subdividido em três fases, sendo a primeira de crescimento inicial, quando a cultura se estabelece. Durante a fase 2 a cultura cresce exponencialmente, e durante a fase 3, nomeada fase de degeneração, ocorre aumento ocasional de células tetraplóides e os núcleos celulares apresentam-se com aparência incomum. Os seus experimentos mostraram que as cepas de células humanas entram em processo de senescência a partir de certo número de mitoses, independentemente do tempo e da fase em que ficaram congeladas (HAYFLICK, 1965). Este processo sugere que, internamente, as células possuem mecanismo de "contagem", o qual monitora a quantidade acumulada de divisões celulares.

Na década de 70, foi observado que a replicação de DNA linear ocorria de forma completa no sentido 5´ para 3´, até o fim da fita contínua. Entretanto, a cópia da fita descontínua não era sintetizada na íntegra. A replicação da fita descontínua ocorre através de *primers* de RNA, os quais são estendidos e os fragmentos de Okazaki são formados e ligados uns aos outros após a retirada do RNA iniciador. No entanto, conforme demonstrado pela Figura 1, esse processo não permite a síntese da lacuna remanescente após a retirada do último *primer* de RNA e o fim da mesma, o que inviabiliza a formação de um novo fragmento de Okazaki e a replicação total da fita descontínua. À medida que repetidas mitoses ocorrem, esse fragmento não sintetizado também não é sintetizado nas sucessivas divisões celulares. Este processo é chamado de problema do "final da replicação"(Figura 1), em que ocorre o encurtamento progressivo de telômeros (RUDOLPH, 2010).

Figura 1.1 O problema do final da replicação. A fita descontínua é sintetizada a partir de uma série de fragmentos de Okasaki, os quais necessitam *primers* iniciadores para sua formação. Ao final da fita descontínua não é possível preencher o espaço deixado entre o último fragmento de Okasaki e o fim da sequencia de telômeros (SHAY; WRIGHT, 2000).

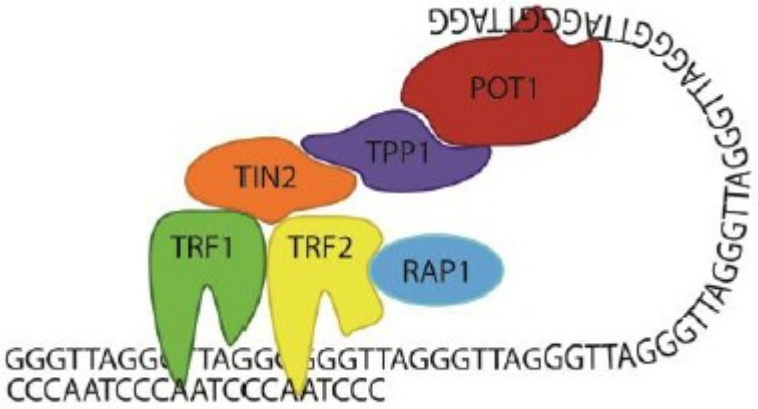


Fonte: Adaptada de: Shay, J. W., & Wright, W. E. (2000). Hayflick, his limit, and cellular ageing. *Nature reviews Molecular cell biology*, *1*(1), 72–76.

À extremidade dos cromossomos lineares estão localizados os telômeros, que são nucleoproteínas complexas, que apresentam repetições de DNA ricas em G, circundadas por diversas proteínas específicas (TRF1, TRF2, Tin2, Rap1, TPP1, POTI), compondo um complexo de polipeptídeos que interagem entre si e com as repetições (Figura 2). As porções de DNA de fita dupla são interligadas pela TRF1 e TRF 2. A primeira apresenta papel central na proteção do telômero, sua superexpressão está relacionada ao encurtamento gradual do telômero, enquanto sua ausência provoca alongamento telomérico. Já a TRF2 está envolvida na sinalização de dano no DNA telomérico. A perda de TRF2 ativa a ATM, proteíno-quinase ativada para reparo de DNA diante de quebras de fita dupla, além da Apollo nuclease, também envolvida em processos de reparo (BLASCO, 2007). A extremidade 3` do telômero é circundada pela POT1, e duas outras proteínas, TIN2 e TPP1, que atuam como ligadoras da

POT1 e TRF1 e 2, fazendo com que a extremidade dos telômeros assumam o formato de laço (*t-loop)*. Este perfil de finalização telomérica permite que as extremidades dos cromossomos sejam distinguidas de quebras de DNA. Este complexo, assim como as proteínas envolvidas no reparo de DNA atuam protegendo a porção telomérica (HOHENSINNER; GORONZY; WEYAND, 2011).

Figura 1.2 Complexo de proteínas teloméricas - *Shelterin Complex*



Fonte: Hohensinner, P. J., Goronzy, J. J., & Weyand, C. M. (2011). Telomere dysfunction, autoimmunity and aging. *Aging and disease*, *2*(6), 524.

Em células humanas telômeros são compostos de repetições da sequência de bases TTAGGG e variam entre 6 a 12 (kb) kilobases de comprimento (AUBERT; LANSDORP, 2008).

A disfunção telomérica pode apresentar consequências como elevados níveis de recombinação de extremidades e fusão cromossômica, padrões de expressão gênica alterados e instabilidade genômica (BLACKBURN, E. H., 2001), indicando a capacidade protetiva dos telômeros.

1.2 TELOMERASE

Para compensar o encurtamento telomérico, a replicação dos telômeros é mediada pela transcriptase reversa nomeada telomerase (TERT - Telomerase Reverse Transcriptase), enzima composta por um complexo de ribonucleoproteínas, a qual apresenta uma fita molde de RNA intrínseco, que desempenha papel essencial. A telomerase reconhece a terminação 3` - OH na extremidade do cromossomo e acrescenta a sequencia telomérica por meio do componente intrínseco de RNA, nomeado Terc (Telomerase RNA component). Bloqueando-se a disponibilidade da Terc, a função da telomerase estará comprometida (HARLEY; FUTCHER; GREIDER, 1990). A telomerase está presente nas células tronco, no entanto, as células somáticas são carentes da enzima, fazendo com que estejam sujeitas a um ciclo vital finito (HARLEY; FUTCHER; GREIDER, 1990).

Os experimentos com cultura celular de Hayflick apontaram para a finitude do potencial de replicação celular. No entanto, quando a telomerase é inserida no procedimento, o comprimento telomérico, independente da fase da cultura, é estabilizado e o crescimento de aberrações cromossômicas era estancado (HAYFLICK, 1965; HAYFLICK; MOORHEAD; OTHERS, 2006). Este processo sugere a ação da telomerase como mecanismo base para estabilização do comprimento telomérico (HARLEY; FUTCHER; GREIDER, 1990). O fato de estar ativa em células-tronco e durante o desenvolvimento inicial assegura que o encurtamento de telômeros seja contido e que seu comprimento seja constante, favorecendo uma replicação celular adequada nessa fase do desenvolvimento. Nas células somáticas, à medida que o encurtamento ocorre, os telômeros configuram-se ineficazes na manutenção das propriedades protetivas e regulatórias nas extremidades dos cromossomos (JIANG *et al.*, 2008).

À medida que o encurtamento telomérico ocorre a um nível crítico, inviabilizando a função e estrutura adequada do telômero, sinalizações de reparo ocorrem. Esta função é

exercida principalmente pela proteína TRF2 e evita que células danificadas ou em risco, permaneçam em atividade replicativa (BLASCO, 2007). Assim, quando o telômero encurta criticamente, a fusão e quebra cromossômica são favorecidas, o que consequentemente gera morte celular apoptótica. Com o risco de apoptose aumentado, a proliferação celular é diminuída, o que compromete a renovação celular (BLASCO, 2007) , viabilizando disfunção tecidual e o envelhecimento ou senescência da célula. Através desta dinâmica, o comprimento telomérico é considerado relógio biológico para envelhecimento celular. O aceleramento do encurtamento de telômeros está associado à redução do tempo de vida em modelos animais (WONG *et al.*, 2003). Este achado é corroborado em estudo com humanos, de modo que a quantidade de telômeros curtos em células mononucleares sanguíneas periféricas (PBMCs) aumenta de acordo com a idade (CANELA *et al.*, 2007).

1.3 RELAÇÃO ENTRE ESTRESSE E TELÔMEROS

Os índices de encurtamento podem variar segundo algumas características da cultura celular, como a viabilidade da polimerase α posicionar o *primer* mais distal ou mais perto do fim do cromossomo, como ocorre com fibroblastos humanos, que têm perda de 10 a 20bp por duplicação de sua população (TAN, 1999). Além disso, o índice de encurtamento pode diferir segundo comprimento telomérico inicial, influências genéticas (BROER *et al.*, 2013) ou ainda variar segundo os próprios níveis de replicação celular o que depende do tecido tratado. No entanto, além desses fatores, a heterogeneidade no encurtamento de um mesmo tecido em condições diferentes coloca em evidência influências do ambiente celular sobre a dinâmica telomérica (VON ZGLINICKI, [S.d.]).

Agentes oxidativos promovem danos ao DNA genômico, possibilitando apoptose e outros mecanismos de controle independente dos telômeros, o que também estaria associado a uma contenção da proliferação da cultura celular. Estas evidências geram questionamento

sobre a influência agentes oxidativos sobre a dinâmica de encurtamento de telômeros. Em sua revisão, (VON ZGLINICKI, 2002) verifica a independência ou a sobreposição entre senescencia replicativa, resultado do problema do final da replicação, e senescencia induzida por estresse, comparando o comprimento telomérico em relação a tratamentos de indução de estresse, tratamentos antioxidativos e cultura celular controle. Em condições de estresse oxidativo elevado, apesar de que telômeros são estruturas de DNA ricas em G, e são altamente sensíveis a dano por alquilação, irradiação ultravioleta (UV), e estresse oxidativo, danos no DNA poderiam ocorrer também em outras regiões do DNA. Nestas condições os danos são fortes o suficiente para deter o crescimento celular e não necessariamente influenciar o comprimento telomérico. Dessa forma, além da indução de quebras de fita dupla, em condições moderadas de estresse, foi verificada uma deficiência no reparo de quebras de fita única, além de uma maior frequência desse dano quando comparada a outras porções do genoma (PETERSEN; SARETZKI; ZGLINICKI, 1998; VON ZGLINICKI; PILGER; SITTE, 2000). Além destas evidencias, outros estudos demonstraram correlação positiva entre a frequência de quebra de fita única e índice de encurtamento telomérico. Dessa forma, senescência celular derivada da dinâmica telomérica depende não só dos níveis de divisão celular, através do problema do final da replicação, como das condições da cultura celular, como o equilíbrio entre estresse oxidativo e defesa antioxidante (VON ZGLINICKI, 2002).

1.4 TRANSTORNO AFETIVO BIPOLAR E ESTRESSE OXIDATIVO

O Transtorno Afetivo Bipolar (TAB) é um transtorno caracterizado por episódios recorrentes de mania ou hipomania e depressão. Há dois subtipos principais do transtorno, sendo que o tipo I é caracterizado por um ou mais episódios maníacos ou mistos e, com freqüência, episódios depressivos. Já o tipo II envolve episódios de depressão e presença de

pelo menos um episódio de hipomania (ASSOCIATION; DSM-IV, 2000). De acordo com dados da Organização Mundial da Saúde (WHO, 2005), o TAB está entre as dez doenças que mais levam à incapacidade no exercício das tarefas laborativas.

Os pacientes com TAB tipo I apresentam pior prognóstico do que os com TAB tipo II, sendo rebaixado o funcionamento global e superior o declínio cognitivo, quantidade de episódios (CAVANAGH *et al.*, 2002),e envolvimento em situações de risco.

O TAB representa perfil clínico com presença de prejuízos funcionais e envolvimento em situações com elevada carga de estresse. A natureza do estresse apresenta-se como respostas do organismo rumo à adaptação ao meio (KORTE *et al.*, 2005). Este estudo apresenta o conceito de alostase para definição de "estresse". Alostase é concebida como processo adaptativo de manutenção ativa da estabilidade frente a mudanças (STERLING; EYER, 1988). A idéia central é que existe um custo ao organismo caso os mediadores da alostase (hormônios adrenais, neurotransmissores, alterações respiratórias, cardiovasculares, das imunológicas e desequilíbrio oxidativo) sejam ativados de forma muito freqüente e disfuncional (KORTE *et al.*, 2005). Este custo é chamado de carga alostática e confere vulnerabilidade ao indivíduo, sendo resultado de uma exposição crônica ao estresse, situações que demandam mudança ou adaptação.

O estresse oxidativo é considerado um mediador da alostase (KAPCZINSKI *et al.*, [S.d.]). Diversas evidências apontam para aumento de estresse oxidativo em pacientes com TAB. (BENES *et al.*, 2006), em estudo de perfil de expressão gênica, demonstrou a presença de hipoativação de genes associados a processos antioxidativos. Além disso, genes reguladores da função mitocondrial são hipoativados em regiões frontais e hipocampais de pacientes com TAB, acarretando aumento da produção de substâncias reativas ao oxigênio (SRO) (IWAMOTO; BUNDO; KATO, 2005; MACDONALD *et al.*, 2006; SUN *et al.*, 2006). Em relação aos episódios de humor, foram encontrados aumentados níveis de TBARS -

medida de peroxidação lipídica, agente oxidativo, ao longo dos diversos episódios (Andraezza et al., 2997a).

Uma outra possível fonte de estresse oxidativo pode estar relacionada à elevada atividade dopaminérgica durante os episódios maníacos. A Dopamina (DA) é metabolizada via monoamina oxidase (MAO) e produz, entre outras substâncias, H_2O_2 que se não for reduzido por mecanismos antioxidativos, pode reagir com metais de transição como o Ferro e formar radicais hidroxila, o que potencializa os danos ao DNA (KAPCZINSKI *et al.*, 2008).

As evidências sugerem presença de desequilíbrio entre agentes oxidativos e antioxidativos no quadro de TAB. Além disso, a frequencia de doenças clínicas associadas, como as cardiovasculares, diabetes entre outras, apresentam-se em alta frequencia em pacientes com TAB (ANGST *et al.*, 2013). Além da elevada frequencia, as doenças cardiovasculares representam a principal causa de morte entre pacientes bipolares (ANGST *et al.*, 2013). Considerando o encurtamento telomérico como um marcador de exposição ao estresse no nível celular e preditor das condições clínicas citadas, é de grande relevância a verificação de possíveis fatores de risco associados ao encurtamento telomérico.

2. OBJETIVOS

Caracterizar o comprimento telomérico em pacientes com Transtorno Afetivo Bipolar

2.1 OBJETIVOS ESPECÍFICOS

1- Comparar e descrever o comprimento telomérico de pacientes em comparação aos sujeitos controles.

2- Verificar características clinicas inerentes ao TAB associadas à maior vulnerabilidade ao encurtamento telomérico em pacientes com TAB.

3. COMPRIMENTO TELOMÉRICO E TRANSTORNOS PSIQUIÁTRICOS - TELOMERE LENGTH AND PSYCHIATRIC DISORDERS

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Author contributions: The authors contributed equally. All of them performed the research and wrote the paper.

Abstract:

Introduction: Telomere length has been related to oxidative stress and age related diseases. Considering the associations between these aspects and psychiatric disorders, telomere length might be an important vulnerability marker for this population. Therefore, this study aimed to review aspects related to telomere length and quality of life within this public. **Method:** A systematic review was conducted through the combination of *telomere* and the following terms: *depression, bipolar disorder, mood disorders, schizophrenia and anxiety.* **Results:** A total of 35 original studies were included. The results suggested that telomere attrition was present in psychiatric illness, and different risk and protective factors were described among

disorders. Schizophrenia telomere length was associated with hereditary influences. **Discussion:** More studies linking genetic influences, inflammatory and oxidative mechanisms and psychosocial stress across various psychiatric disorders are needed in order to expand the conclusions taken.

Key words: Depression, Bipolar, Schizophrenia, Anxiety, Telomere length.

3.1 INTRODUCTION:

Telomeres are tandem TTAGGG repeats of DNA located at the end of linear chromosomes[1]. Telomeres are supposed to be crucial for maintaining chromosomal integrity, protection from loss of genetic material and end-to-end recombination[2]. Because of its unique features, DNA polymerase does not replicate telomeres during cell replication. This so called "the end replication problem" is in part circumvented by a special enzyme named telomerase, however, it is not always effective, thus leading to shortening of telomeres with time[2].

Leukocyte telomere length is stated by genetic, epigenetic and environmental factors[3–5]. It tends to decrease progressively with inflammation, oxidative stress and especially with aging[6]. Therefore, telomere shortening has been understood as a surrogate index for health status as it reflects cellular injuries and lifespan[7]. Different cell types have unique rates of telomere shortening, because they have distinctive original telomere size, replication speed and telomerase expression. Curiously, telomere shortening speed is higher during early childhood and in the elderly[7–9].

Studies have shown that telomere length is highly dependent on the activity of the enzyme telomerase[2]. It is a ribonuclease nucleoprotein complex mostly expressed in cells with potential to proliferate. Nevertheless, the majority of somatic cells have low telomerase activity. Therefore telomere has been understood as a biological counter[10]. In consonance

with that, an elegant study found an inverse relation between mortality rates determined by any causes and telomere length[11].

In psychiatry, studies have reported decreased telomere length in association with anxiety[12–15] and mood disorders[16–20]. Moreover, short telomeres have been associated with higher levels of psychosocial and early life stressors[21]. Understanding factors associated with telomere shortening in these conditions may shed light into new aspects relevant to longevity and quality of life. Therefore, telomere dynamics in psychiatric disorders is a pertinent question to be answered.

3.2 METHOD:

This study is a systematic review that sought to investigate data regarding telomere length and psychiatric disorders. Inclusion criteria were all articles indexed in PUBMED and SCOPUS database, published until October 2013 and written in English. Review articles were excluded from analyses. Index terms used for search were: *"telomere"* and "*mood disorders"/"bipolar disorder"/"depression"/"schizophrenia"/"anxiety"*.

3.3 RESULTS:

3.3.1 Telomere length and mood disorders

Sixty-six articles were found through combination of terms *"telomere"* and *"depression", "mood disorders", "bipolar disorder"*. Forty-one of them were excluded.

Leukocyte telomere shortening was consistently observed to associated with major depression[16,18,22–25], minor depressive symptoms[19,26–29] and perceived stress[24,30] and wellness[29]. However, some studies did not find these associations[13,14,20,30–33].

Depressive symptoms were considered an additional stressor for caregivers[26], heart failure patients[19,28], chronic pain patients[27], rape victims[14] and dwelling elderly[32]. These

data support the hypothesis that inflammation and stress may shorten telomeres. Recently an index of disease burden, including cardiovascular and pulmonary function, glucose tolerance, kidney function and brain imaging, has been proposed in order to quantify physiologic stress[32]. To look for factors that may induce telomere shortening, a study compared whether this index as well as age could predict leukocyte telomere length. They observed that both parameters have an inverse relation with leukocyte telomere length and that the index itself was capable of predict 35% of of the effect[32]. Therefore, it is suggested that such index is a helpful noninvasive evaluation tool for inferences about how healthy is aging[32]. On the other hand, some studies showed that depression in heart failure patients was not associated[28] and did not predict telomere length variation after five years of follow-up[19]. However, there is evidence suggesting that depressive symptoms in fibromyalgia patients with high levels of pain are predictive of telomere shortening[27].

Six reports sought to determine the relationship between bipolar disorder and telomere length. Five of them used leukocytes for measuring telomeres and one used brain tissue samples[34]. Regarding studies with leukocytes, two suggested that there was no difference in telomeres length in the presence of bipolar disorder[34,35], two other studies did find shorter telomeres in association with the disorder[17,36] and one found longer telomeres in patients treated with Lithium compared to controls[38]. Interestingly, they did not find clinical features related to telomere length, such as rapid cycling subtype[35,37], disorder duration[35,36], number of previous hypomanic episodes[35], or presence of anxiety as comorbidity[17]. However a significant difference in telomere length was observed when patients were stratified by number of depressive episodes[35].

In cross-sectional studies, mood disorders may shorten telomeres by 660bp, after adjusting for age and gender[16]. Considering major depression alone it is estimated a

reduction of 142 bp[23] to 281 bp which would be equivalent to 7 years of accelerated aging according to Wolklowitz[20].

Medication effects over telomere length were not controlled in most of these studies, however, in a Major Depressive sample, medication and dosage was not associated with telomere length[18,24]. Also in bipolar patients, it was observed no difference in telomere length according to medication subtype (lithium, anticonvulsivants, antidepressants and antipsychotics)[36]. Although one study contradict these findings, analyzing the largest bipolar patients sample among bipolar disorder studies, it was found that bipolar patients treated with lithium had longer telomeres (34.5% higher than controls)[37]. Moreover, patients who did not present clinical response to lithium therapy had longer telomere length than controls[37]. Therefore, it was suggested that lithium is a protective factor for telomeres[37]. In consonance with that, another study observed that resilience may protect telomeres in major depressive disorder[23]. Resilience comprises social connections; emotion regulation and health behaviors like sleep and exercise. However, long-term studies were inconclusive about telomere shortening and depression. One cohort study did not find difference in telomere length in depressive patients, although after stratifying patients by age, younger adults with depressive symptoms, assessed at baseline and with two and four years follow-up, showed shorter telomeres[31]. Another study suggested that not depression but anxiety might predict telomere length after two years follow-up in adults[13]. The other two cohort studies have not found any association between telomere length and depressive symptoms in patients with coronary heart disease and elderly[19,29].

For measurement of telomere length, only two studies used cerebellar and prefrontal cortex samples[34,39] while the others rely on leukocyte samples for quantification[13,14,19,24–26,28–33]. Both studies did not find difference in telomere length as measured in cerebellar or dorsolateral prefrontal cortex samples from patients with mood disorders[38,39]. Moreover,

these two studies tried to identify genetic determinants of telomere length. One study aimed to identify SNPs in regulatory regions of certain genes and their association with telomere length in samples of human brain. They did not find any specific variant influencing telomere length[38]. The other study examined expression of stress response genes (C-FOS, MSRA, NEIL1, OGGI, TERF2, SOD1, SOD2, CAT, GPX1) in prefrontal cortex of depressed and non-depressed patients and did not find any significant difference[39].

Studies have suggested that oxidative states caused by chronic stressors may accelerate immunosenescence and telomere shortening. This could be one causative factor of telomere shortening in depressive patients[20]. For instance, it was found a positive association between anti-CMV IgG and percentage of CD8+CD28- (r= 0.33, p= 0.04, n=38) and NK cells (r= 0.41, p= 0.01, n=34). Euthymic type I bipolar patients present significantly higher positive serology for cytomegalovirus than healthy controls, which could foster an inflammatory environment[37]. Another study did not observe differences in inflammatory and oxidative markers in unmedicated depressive patients, although founding correlation between those markers and telomere length in patients[20]. The interface between brain and immune system is complex and has evidence supporting it from the literature. For example, it has been shown that depressive symptoms may change expression of many cytokines like TNF-α and IL-10[26].

Stressful states are also associated with neuroendocrine alterations like increased levels of cortisol indicating an altered hypothalamic-pituitary-adrenal (HPA) axis response. To test whether neuroendocrine response in mood disorders could be responsible for changes in telomere length, depressive patients with low cortisol levels had their leukocyte telomeres measured. They found shorter telomeres in those patients[24]. This is congruent with previous finding that hypocortisolism is associated to some sort of exhaustion of the HPA axis during chronic stress[40]. The mechanisms linking HPA axis alterations and telomere shortening

remains obscure. It has been suggested that low levels of cortisol may lead to an inflammatory state and, therefore, an accelerated telomere shortening[24].

Studies have indicated that mood disorders may lead to disrupted immune responses and consequently to telomere shortening[20]. It is suggested that depression contributes to telomere shortening[20]. However, aging and another disabilities like coronary heart disease and chronic pain are additive to depression and, in the long range, may attenuate the strength with which depression or its symptoms impacts over telomeres. It is possible that bearing an adverse health condition such as mood disorder leads to non-specific alteration on telomere dynamics. The reviewed studies also suggested that the underlying common mechanisms involved in telomere shortening might involve inflammatory and oxidative states.

3.3.2 Telomere length and schizophrenia

Twenty-eight articles were found through combination of terms *"telomere"* and *"schizophrenia"*. Twenty-one of them were excluded and seven were included.

Telomere length decreases during cell replication; this shortening is an important component of cell senescence. Oxidative stress increases the amount of telomeric DNA lost during each replication cycle. There is some evidence that increased oxidation is associated with schizophrenia (SCZ)[41]. This may help explain the pathophysiology of shorter telomere length among psychotic patients.

Experiments have shown that the relation of DNA telomere content (TC) to telomere length is approximated. A score of 100% TC correlates to approximately 10 kb in telomere length. Therefore, each 1% difference in TC reflects approximately a 100-bp difference in telomere length[42].

Some pathological features observed in schizophrenia, like structural brain abnormalities and hypometabolism, are also find in aging related diseases like Alzheimer's

dementia[43]. Moreover, individuals with schizophrenia are prone to diseases associated with aging, including diabetes and cardiovascular complications[44,45], as well as a shorter natural lifespan than the general population[46]. Many factors are contributors, including increased rates of suicide, poor health habits, and smoking. Antipsychotic and antidepressant medications, which are widely used by patients with schizophrenia, are also associated with adverse outcomes like weight gain, diabetes, and hypertension [44].

Assuming that leukocyte telomere length may be a biomarker for exposure to stress, short telomeres would be expected in patients with SCZ since these individuals experience high levels of stress. Several studies have been undergone aiming to evaluate telomere length of patients with schizophrenia[47–49]. However, there are many confounding variables hard to control.

One large study found that telomere length was significantly longer in patients with SCZ[47]. The result remained the same even after adjustment for smoking. This result is consistent with another study that investigated the association between telomere length and history of psychiatric disorder and prescription of psychotropic medication[50]. They observed that hospitalized psychiatric patients, receiving psychotropic medication, displayed longer telomere length compared to healthy individuals. The authors hypothesized that psychotropic medications may have antioxidative effects and thus prevent telomere attrition[50]. Since all hospitalized patients on the study received psychotropic medication, this could have contributed to this finding.

One interesting finding is that advanced paternal age is associated with increased telomere length of the offspring after birth and during their entire life[4,32,51–54]. Curiously, paternal age has also been identified as a consistent risk factor for SCZ[55]. Studies suggest that the underlying mechanism for this phenomenon is that the number of new mutations increases with paternal age, which in turn increases the risk for SCZ[55]. Therefore, long

telomeres observed in schizophrenic patients may be a marker of parental age at the time of conception rather than a causal factor for the disease. However, this has to be replicated by new studies.

Whether telomere length variations is a cause or a consequence of certain diseases remains a matter of debate[56]. The finding of longer telomere length in schizophrenic patients[47] may represent either a consequence of or a necessary condition for SCZ development[47]. Longitudinal study of telomere length has indicated that, besides age, the most powerful predictor of shorter telomere length is their baseline length[57].

One case-control study did not find differences in telomere length between schizophrenic patients and healthy individuals[34]. As reported recently, self reported consanguinity was more frequent among the SCZ cases[58]. Using Pearson correlation test, there was no significant correlation between telomere length and DNA based inbreeding coefficient values among controls. However, a significant correlation between telomere length and DNA based inbreeding coefficient values was noted among cases. ANCOVA analyses using telomere length as the dependent variable, with age, sex and case/control status as predictors did not indicate any significant group-wise differences. Linear regression analyses using telomere length as the dependent variable, with age and case/control status, gender and inbreeding coefficients as predictors also did not indicate any significant group-wise differences. On the other hand, a significant association with inbreeding was observed.

One study measured telomere length from genomic DNA extracted from 155 brain samples (gray matter of cerebellum) from patients of European ancestry with psychiatric disorders [34]. They measured mean telomere length by quantitative PCR with some modifications[59]. No difference was observed in mean telomere length between schizophrenic, bipolar and depressive patients and controls. Also, age, gender, medication and drug use had no effect on mean telomere length.

Conversely, one study observed that psychotic patients had significantly decreased mean DNA telomere content compared with control subjects[42]. Although telomere length is related to gender[57], they did not find differences of telomere content between men and women; the two groups were also very similar regarding to gender composition. Another study found significantly shorter telomeres in the schizophrenic patients compared to family members and controls[48]. Moreover, study suggests that disease severity correlates directly with telomere length[49].

As stated before, telomere length is quite dependable on telomerase. To test whether telomerase activity is an important factor determining telomere length in psychiatric disorder, one group studied lymphocytes telomerase in schizophrenic patients. They found considerable variation in lymphocyte telomerase levels among patients and controls, suggesting that this enzymatic activity is subject to physiological fluctuation in humans[60]. No correlation between telomerase activity and age, or association between telomerase activity and sex was observed.

The highest telomerase levels were found in unaffected relatives of individuals with schizophrenia, but these levels did not differ significantly from related family members of patients with schizophrenia. The lowest telomerase levels were found in individuals with schizophrenia. When all unaffected individuals, controls and family, were taken together and compared with all individuals with schizophrenia, a nominally significant reduction in telomerase activity was observed in patients with schizophrenia[60].

Yu *et al.*(2004)[49] analyzed telomeric DNA from patients with schizophrenia who responded differently to antipsychotics. They observed a significant telomere shortening in peripheral blood leukocytes from patients with schizophrenia who were poor responders to antipsychotics and no difference between good responders and the control subjects.

It has been hypothesized that telomere shortening might serve as a biological clock that could count mitotic time and indicate for cell senescence in culture. Loss of replicative capacity leads to cell growth arrest, which occurs after accumulated doubling populations and as a consequence of cell stressors such as mild chronic oxidative stress. Telomere shortening has been seen in cell cultures subjected to different stressors such as chronic hyperoxia. This was also observed in fibroblasts from patients with Fanconi's anemia and in peripheral blood leukocytes from patients with mitochondrial disorders; both diseases result in increased oxidative stress[61].

Several studies have demonstrated that disruption of antioxidant capacity may contribute to an oxidative state in schizophrenia. It has been shown that oxidative stress might cause mitochondrial dysfunction and altered brain metabolism in schizophrenia and shortening of telomeres[49]. This was supported by a report showing that oxidative DNA damage was 10 times greater in postmortem hippocampi of elderly patients with "poor-outcome" schizophrenia[62]. Further studies are needed to establish the status and role of oxidative stress in the pathogenesis of schizophrenia.

3.3.3 Telomere length and anxiety disorders

The search of terms "telomere "and "anxiety" resulted in twenty-three articles, however, many of them were excluded based because their data did not fit standardized definitions of anxiety disorders.

In the study of Kananen et al. (2010)[12], they compared telomere length in 282 individuals with anxiety disorder and 653 matched controls from Finland. A shorter telomere length was found among older individuals with anxiety. In this study an interesting finding was the fact that anxious individuals with history of early life stress, in special severe illness during childhood, were associated with shorter telomeres and diagnosis of anxiety in

adulthood[12]. In consonance, a later work, Okereke et al. (2012)[63] identified shorter telomeres in a cross-sectional analyses of women with phobic anxiety and body mass index higher than 25. Another study evaluated the association between anxiety and depressive disorders and telomere length. Only anxiety was associated with shorter telomeres independent of any environmental factor[13]. More studies are needed to fully understand the effect of anxiety over the length of telomeres.

3.4 DISCUSSION:

We sought to identify publications linking psychiatric disorders and telomere lengths. Telomere attrition seems to be determined by various aspects of psychiatric disorders, for example, psychosocial stress. The mechanisms underlying the telomere shortening phenotype was most studied in depression and a clear involvement of altered HPA axis stress response[24], inflammation[20,26,36] and oxidative stress[20,26] were observed.

Regarding the dynamics of telomere shortening in Schizophrenia, some studies with large samples highlighted the presence of longer telomeres in patients[47,50]. Moreover, since there is a positive correlation between consaguinity[58] and paternal age at conception and telomere length[4,47–51], it is relevant to consider telomere baseline length for the analyzes of those studies. Noteworthy, according to Nordfjall[4] baseline longer telomere length are associated with greater rates of attrition.

Among psychiatric disorders, telomere shortening risk factors include: psychotic symptoms severity[42], poor response to treatment[49], illness severity[49], for Schizophrenia; number of previous depressive episodes for Bipolar Disorder[35]; and adverse childhood events in anxiety disorders[12]. In schizophrenia, a protective factor may include the use of psychotropic drugs that is possibly related to antioxidant activity[50]. In case of bipolar disorders, lithium is also associated with longer telomere length[38]. Despite lack of evidence

for protection of telomere by medications in anxiety or depression, it is suggested that resilience may be an important protection factor in depressive patients[23].

Whether telomere length is cause or consequence of psychiatric disorders remains unclear[56]. Another important aspect is that psychiatric illnesses are quite heterogeneous conditions that hampers generalizations. Nevertheless, it is relevant to continue the efforts to describe and determine aspects related to telomere attrition in psychiatric context. New findings can shed light into therapeutic targets and mechanisms of disease that may ultimately lead to therapeutic benefits to patients.

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3.5 REFERENCES:

1. Blackburn EH. Telomere states and cell fates. Nature. 2000;408(6808):53–6. [PMID:11081503 DOI:10.1038/35040500]

2. Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. Febs Letters. 2005;579(4):859–62. [PMID: 15680963 DOI: 10.1016/j.febslet.2004.11.036]

3. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. American journal of human genetics. 1994;55(5):876. [PMID: 7977349]

4. Nordfjäll K, Svenson U, Norrback K-F, Adolfsson R, Roos G. Large-scale parent–child comparison confirms a strong paternal influence on telomere length. European Journal of Human Genetics. 2009;18(3):385–9. [PMID:19826452 DOI: 10.1038/ejhg.2009.17]

5. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nature genetics. 2013;45(4):422–7. [PMID: 2353573 DOI: 10.1038/ng.2528]

6. Svenson U, Nordfjäll K, Baird D, Roger L, Osterman P, Hellenius M-L, et al. Blood cell telomere length is a dynamic feature. PloS one. 2011;6(6):e21485. [PMID:21720548 DOI: 10.1371/journal.pone.0021485]

7. Aviv A, Valdes AM, Spector TD. Human telomere biology: pitfalls of moving from the laboratory to epidemiology. International journal of epidemiology. 2006;35(6):1424–9. [PMID: 16997848 DOI: 10.1093/ije/dyl169]

8. Iwama H, Ohyashiki K, Ohyashiki JH, Hayashi S, Yahata N, Ando K, et al. Telomeric length and telomerase activity vary with age in peripheral blood cells obtained from normal individuals. Human genetics. 1998;102(4):397–402. [PMID:960023 DOI: 10.1007/s004390050711]

9. Blackburn EH. Switching and signaling at the telomere. Cell. 2001;106(6):661–73. [PMID:11572773 DOI: 10.1016/S0092-8674(01)00492-5

10. Aubert G, Lansdorp PM. Telomeres and aging. Physiological reviews. 2008;88(2):557–79. [PMID:22661914 DOI: 0.1152/physrev.00026.2007]

11. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. The Lancet. 2003;361(9355):393–5. [PMID: 12573379 DOI: 10.1016/S0140-6736(03)12384-7]

12. Kananen L, Surakka I, Pirkola S, Suvisaari J, Lönnqvist J, Peltonen L, et al. Childhood adversities are associated with shorter telomere length at adult age both in individuals

with an anxiety disorder and controls. PLoS ONE. 2010;5(5):e10826. [PMID: 20520834 DOI: 10.1371/journal.pone.0010826

13. Hoen PW, Rosmalen JGM, Schoevers RA, Huzen J, Van der Harst P, De Jonge P. Association between anxiety but not depressive disorders and leukocyte telomere length after 2 years of follow-up in a population-based sample. Psychol Med. 2013;43(4):689–97. [PMID: 22877856 DOI: 10.1017/S0033291712001766]

14. Malan S, Hemmings S, Kidd M, Martin L, Seedat S. Investigation of telomere length and psychological stress in rape victims. Depress Anxiety. 2011;28(12):1081–5. [PMID: 22065550 DOI: 10.1002/da.20903]

15. O'Donovan A, Epel E, Lin J, Wolkowitz O, Cohen B, Maguen S, et al. Childhood trauma associated with short leukocyte telomere length in posttraumatic stress disorder. Biological psychiatry. 2011;70(5):465–71. [PMID: 21489410 DOI: 10.1016/j.biopsych.2011.01.035]

16. Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, et al. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol. Psychiatry. 2006;60(5):432–5. [PMID: 16581033 DOI: 10.1016/j.biopsych.2006.02.004]

17. Lung F-W, Chen NC, Shu B-C. Genetic pathway of major depressive disorder in shortening telomeric length. Psychiatr. Genet. 2007;17(3):195–9. [PMID: 17417064 DOI:10.1097/YPG.0b013e32808374f6]

18. Hartmann N, Boehner M, Groenen F, Kalb R. Telomere length of patients with major depression is shortened but independent from therapy and severity of the disease. Depress Anxiety. 2010;27(12):1111–6. [PMID:2105333 DOI: 10.1002/da.20749]

19. Hoen PW, De Jonge P, Na BY, Farzaneh-Far R, Epel E, Lin J, et al. Depression and leukocyte telomere length in patients with coronary heart disease: data from the Heart and Soul Study. Psychosom Med. 2011;73(7):541–7. [PMID: 21597035 DOI: 10.1097/PSY.0b013e31821b1f6e]

20. Wolkowitz OM, Mellon SH, Epel ES, Lin J, Dhabhar FS, Su Y, et al. Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress--preliminary findings. PLoS ONE. 2011;6(3):e17837. [PMID: 20376837 DOI: 10.1002/da.20686]

21. Price LH, Kao H-T, Burgers DE, Carpenter LL, Tyrka AR. Telomeres and early-life stress: an overview. Biol. Psychiatry. 2013;73(1):15–23. [PMID:22831981 DOI: 10.1016/j.biopsych.2012.06.025]

22. Garcia-Rizo C, Fernandez-Egea E, Miller BJ, Oliveira C, Justicia A, Griffith JK, et al. Abnormal glucose tolerance, white blood cell count, and telomere length in newly diagnosed, antidepressant-naïve patients with depression. Brain Behav. Immun. 2013;28:49–53. [PMID: 23207109 DOI: 10.1016/j.bbi.2012.11.009]

23. Puterman E, Epel ES, Lin J, Blackburn EH, Gross JJ, Whooley MA, et al. Multisystem resiliency moderates the major depression-telomere length association: findings from the Heart and Soul Study. Brain Behav. Immun. 2013;33:65–73. [PMID:23727245 DOI: 10.1016/j.bbi.2013.05.008]

24. Wikgren M, Maripuu M, Karlsson T, Nordfjäll K, Bergdahl J, Hultdin J, et al. Short telomeres in depression and the general population are associated with a hypocortisolemic state. Biol. Psychiatry. 2012;71(4):294–300. [PMID: 22055018 DOI: 10.1016/j.biopsych.2011.09.015

25. Teyssier J-R, Chauvet-Gelinier J-C, Ragot S, Bonin B. Up-regulation of leucocytes genes implicated in telomere dysfunction and cellular senescence correlates with depression and anxiety severity scores. PloS one. 2012;7(11):e49677. [PMID: 23185405 DOI: 10.1371/journal.pone.0049677]

26. Damjanovic AK, Yang Y, Glaser R, Kiecolt-Glaser JK, Nguyen H, Laskowski B, et al. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. The Journal of Immunology. 2007;179(6):4249–54. [PMID:17785865]

27. Hassett AL, Epel E, Clauw DJ, Harris RE, Harte SE, Kairys A, et al. Pain is associated with short leukocyte telomere length in women with fibromyalgia. J Pain. 2012;13(10):959–69. [PMID: 22877856 DOI: 10.1017/S0033291712001766]

28. Huzen J, Van der Harst P, De Boer RA, Lesman-Leegte I, Voors AA, Van Gilst WH, et al. Telomere length and psychological well-being in patients with chronic heart failure. Age Ageing. 2010;39(2):223–7. [PMID: 20085922 DOI: 10.1093/ageing/afp256]

29. Rius-Ottenheim N, Houben JMJ, Kromhout D, Kafatos A, Van der Mast RC, Zitman FG, et al. Telomere length and mental well-being in elderly men from the Netherlands and Greece. Behav. Genet. 2012;42(2):278–86. [PMID: 21870178 DOI: 10.1007/s10519-011-9498-6]

30. Georgin-Lavialle S, Moura DS, Bruneau J, Chauvet-Gélinier J-C, Damaj G, Soucie E, et al. Leukocyte telomere length in mastocytosis: Correlations with depression and perceived stress. Brain Behav. Immun. 2013; [PMID: 23917070 DOI: 10.1016/j.bbi.2013.07.009]

31. Phillips AC, Robertson T, Carroll D, Der G, Shiels PG, McGlynn L, et al. Do symptoms of depression predict telomere length? Evidence from the west of Scotland twenty-07 study. Psychosom Med. 2013;75(3):288–96. [PMID: 23513237 DOI: 10.1097/PSY.0b013e318289e6b5]

32. Sanders JL, Fitzpatrick AL, Boudreau RM, Arnold AM, Aviv A, Kimura M, et al. Leukocyte telomere length is associated with noninvasively measured age-related disease: The Cardiovascular Health Study. J. Gerontol. A Biol. Sci. Med. Sci. 2012;67(4):409–16. [PMID: 21934123 DOI: 10.1093/gerona/glr173]

33. Shaffer JA, Epel E, Kang MS, Ye S, Schwartz JE, Davidson KW, et al. Depressive symptoms are not associated with leukocyte telomere length: findings from the Nova Scotia Health Survey (NSHS95), a population-based study. PLoS ONE. 2012;7(10):e48318. [PMID: 23133583 DOI: 10.1371/journal.pone.0048318]

34. Zhang D, Cheng L, Craig DW, Redman M, Liu C. Cerebellar telomere length and psychiatric disorders. Behav. Genet. 2010;40(2):250–4. [PMID: 20127402 DOI: 10.1007/s10519-010-9338-0]

35. Mansour H, Chowdari K, Fathi W, Elassy M, Ibrahim I, Wood J, et al. Does telomere length mediate associations between inbreeding and increased risk for bipolar I disorder and schizophrenia? Psychiatry Res. 2011;188(1):129–32. [PMID: 21300409 DOI: 10.1016/j.psychres.2011.01.010]

36. Elvsåshagen T, Vera E, Bøen E, Bratlie J, Andreassen OA, Josefsen D, et al. The load of short telomeres is increased and associated with lifetime number of depressive episodes in bipolar II disorder. J Affect Disord. 2011;135(1-3):43–50. [PMID: 21880373 DOI: 10.1016/j.jad.2011.08.006]

37. Rizzo LB, Do Prado CH, Grassi-Oliveira R, Wieck A, Correa BL, Teixeira AL, et al. Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. Bipolar Disord. 2013; [PMID: 24021055 DOI: 10.1111/bdi.12121]

38. Martinsson L, Wei Y, Xu D, Melas PA, Mathé AA, Schalling M, et al. Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres. Transl Psychiatry. 2013;3:e261. [PMID: 23695236 DOI: 10.1038/tp.2013.37]

39. Teyssier J-R, Ragot S, Chauvet-Gélinier J-C, Trojak B, Bonin B. Expression of oxidative stress-response genes is not activated in the prefrontal cortex of patients with depressive disorder. Psychiatry Res. 2011;186(2-3):244–7.[PMID: 20800905 DOI: 10.1016/j.psychres.2010.07.030]

40. Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology. 2000;25(1):1–35. [PMID: 10633533 DOI: 10.1016/S0306-4530(99)00035-9]

41. Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang J-J, Griffin JL, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Molecular psychiatry. 2004;9(7):684–97.[PMID:15098003]

42. Fernandez-Egea E, Bernardo M, Heaphy CM, Griffith JK, Parellada E, Esmatjes E, et al. Telomere length and pulse pressure in newly diagnosed, antipsychotic-naive patients with nonaffective psychosis. Schizophr Bull. março de 2009;35(2):437–42. [PMID: 19279086 DOI: 10.1093/schbul/sbn169]

43. Buchsbaum MS, Hazlett EA. Functional brain imaging and aging in schizophrenia. Schizophrenia research. 1997;27(2):129–41. [PMID: 9416643 DOI: 10.1016/S0920-9964(97)00076-5]

44. Hennekens CH, Hennekens AR, Hollar D, Casey DE. Schizophrenia and increased risks of cardiovascular disease. American heart journal. 2005;150(6):1115–21. [PMID: 16338246 DOI: 10.1016/j.ahj.2005.02.007]

45. Mitchell AJ, Malone D. Physical health and schizophrenia. Current Opinion in Psychiatry. 2006;19(4):432–7. [DOI: 10.1097/01.yco.0000228767.71473.9e]

46. Tsuang MT, Woolson RF. Excess mortality in schizophrenia and affective disorders: do suicides and accidental deaths solely account for this excess? Archives of General Psychiatry. 1978;35(10):1181. [PMID: 697536 DOI: 10.1001/archpsyc.1978.01770340031002]

47. Nieratschker V, Lahtinen J, Meier S, Strohmaier J, Frank J, Heinrich A, et al. Longer telomere length in patients with schizophrenia. Schizophr. Res. 2013;149(1-3):116–20. [PMID: 23870621 DOI: 10.1016/j.schres.2013.06.043]

48. Kao H-T, Cawthon RM, Delisi LE, Bertisch HC, Ji F, Gordon D, et al. Rapid telomere erosion in schizophrenia. Mol. Psychiatry. 2008;13(2):118–9. [PMID:18202693 DOI: 10.1038/sj.mp.4002105]

49. Yu W-Y, Chang H-W, Lin C-H, Cho C-L. Short telomeres in patients with chronic schizophrenia who show a poor response to treatment. J Psychiatry Neurosci. maio de 2008;33(3):244–7. [PMID: 18592039]

50. Savolainen K, Eriksson JG, Kananen L, Kajantie E, Hovatta I, Lahti M, et al. Combination of early life stress and traumatic experiences across the lifespan are associated with shorter leukocyte telomere length in later adulthood: the Helsinki Birth Cohort Study. European Journal of Psychotraumatology. 3.

51. Unryn BM, Cook LS, Riabowol KT. Paternal age is positively linked to telomere length of children. Aging cell. 2005;4(2):97–101. [PMID: 15771613 DOI: 10.1111/j.1474-9728.2005.00144.x]

52. Njajou OT, Cawthon RM, Damcott CM, Wu S-H, Ott S, Garant MJ, et al. Telomere length is paternally inherited and is associated with parental lifespan. Proceedings of the National Academy of Sciences. 2007;104(29):12135–9. [PMID: 17623782 DOI: 10.1073/pnas.0702703104]

53. Eisenberg DT, Hayes MG, Kuzawa CW. Delayed paternal age of reproduction in humans is associated with longer telomeres across two generations of descendants. Proceedings of the National Academy of Sciences. 2012;109(26):10251–6. [PMID: 22689985 DOI: 10.1073/pnas.1202092109]

54. Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, et al. Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. European Journal of Human Genetics. 2013; [PMID: 23321625 DOI: 10.1038/ejhg.2012.303]

55. Perrin MC, Brown AS, Malaspina D. Aberrant epigenetic regulation could explain the relationship of paternal age to schizophrenia. Schizophrenia bulletin. 2007;33(6):1270–3. [PMID: 17712030 DOI: 10.1093/schbul/sbm093]

56. De Meyer T, Rietzschel ER, De Buyzere ML, Van Criekinge W, Bekaert S. Studying telomeres in a longitudinal population based study. Frontiers in bioscience: a journal and virtual library. 2008;13:2960. [PMID: 17981769 DOI: 10.2741/2901]

57. Farzaneh-Far R, Lin J, Epel E, Lapham K, Blackburn E, Whooley MA. Telomere length trajectory and its determinants in persons with coronary artery disease: longitudinal findings from the heart and soul study. PloS one. 2010;5(1):e8612. [PMID:20072607 DOI: 10.1371/journal.pone.0008612]

58. Mansour H, Fathi W, Klei L, Wood J, Chowdari K, Watson A, et al. Consanguinity and increased risk for schizophrenia in Egypt. Schizophrenia research. 2010;120(1):108–12. [PMID: 20435442 DOI: 10.1016/j.schres.2010.03.026]

59. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic acids research. 2002;30(10):e47–e47. [PMID: 12000852 DOI: 10.1093/nar/30.10.e47]

60. Porton B, Delisi LE, Bertisch HC, Ji F, Gordon D, Li P, et al. Telomerase levels in schizophrenia: a preliminary study. Schizophr. Res. 2008;106(2-3):242–7. [PMID: 18829263 DOI: 10.1016/j.schres.2008.08.028]

61. Callén E, Samper E, Ramírez MJ, Creus A, Marcos R, Ortega JJ, et al. Breaks at telomeres and TRF2-independent end fusions in Fanconi anemia. Human molecular genetics. 2002;11(4):439–44. [PMID:11854176]

62. Nishioka N, Arnold SE. Evidence for oxidative DNA damage in the hippocampus of elderly patients with chronic schizophrenia. The American journal of geriatric psychiatry. 2004;12(2):167–75. [PMID:15010346]

63. Okereke OI, Prescott J, Wong JY, Han J, Rexrode KM, De Vivo I. High phobic anxiety is related to lower leukocyte telomere length in women. PloS one. 2012;7(7):e40516. [PMID: 22808180 DOI: 10.1371/journal.pone.0040516]

4. ANÁLISE DA HETEROGENEIDADE DO ENCURTAMENTO TELOMÉRICO NO TRANSTORNO AFETIVO BIPOLAR – ANALYSES OF HETEROGENEITY OF TELOMERE ATTRITION IN BIPOLAR DISORDER

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Abstract: Objectives: Investigate whether bipolar disorder (BD) and its clinical specificities are associated with telomere shortening. **Methods:** Eighty five BD patients and 95 healthy controls were paired for age, sex and educational level. Volunteers were submitted to a psychiatric interview and clinical evaluation. Groups were compared as a whole sample and within specific telomere ranges (short/medium and long telomeres). Intrapatients group comparison involved type of BD and comorbidities. It was performed a Real Time Quantitative PCR in order to verify telomere length in leukocytes. **Results:** Patients presented shorter telomeres when compared to controls (p<0,001), athough no specific clinical features

was stablished as a risk factor. Telomere shortening did not occur homogeneously along BD group, since BD was predictive of telomere length in a specific group of subjects (β = -0.416, p<0,001, R^2 = 0.432). **Conclusions:** Taken together these data suggest that bipolar disorder is directly associated with reduced telomere length, a feature similar to what is seen in aging but with a heterogeneous expression among bipolar patients. This result points to the presence of protective factors withing this subgroup of BD patients.

Key words: Bipolar Disorder, Telomere length, Stress, Aging.

4.1. INTRODUCTION

Telomeres are tandem TTAGGG repeats of DNA located at the end of linear chromosomes (BLACKBURN, ELIZABETH H., 2000). They are crucial for maintaining chromosomal integrity and to protect them from loss of genetic material and end-to-end recombination (BLACKBURN, ELIZABETH H., 2005). Due to its unique features, the enzyme named telomerase is in charge of replicating telomeric regions. However, in most human somatic tissues, telomerase activity is insufficient to avoid telomere erosion over time (CHAN; BLACKBURN, 2004). Consequently, telomeres shorten after each cell division and may be used as a surrogate marker of biological aging (BLACKBURN, ELIZABETH H., 2005). For instance, studies have suggested that telomere shortening may be associated with aging related diseases such as psychiatric disorders, cardiovascular diseases and diabetes (Brouilette et al., 2003) (Huzen et al., 2010) (Salpea et al., 2010).

Telomere length and telomerase activity are influenced by several factors including polymorphisms on the gene encoding the telomerase reverse transcriptase, body mass index status and cigarette consumption (BABIZHAYEV *et al.*, 2011; BARRETT; RICHARDSON, 2011; VALDES *et al.*, 2005). It has been hypothesized that these conditions and oxidative and inflammatory stress states may trigger cellular responses that ultimately lead to senescence

and telomere shortening (ZANNI; WICK, 2011). Therefore, it has been suggested that telomere length may be the result of response to cellular stress (von Zglinicki, 2005).

Stress is defined as a threat to homeostasis. An organism responds to it by making efforts to return to its previous state (MCEWEN; STELLAR, 1993). However, chronic stress exposure and its accompanying cellular stress response may be dysfunctional (MCEWEN, 2003). For example, long term depression may lead to a state of hypocortisolism (WIKGREN *et al.*, 2012). Moreover, studies have shown that high levels of pro-inflammatory cytokines during a depressive episode are associated with telomere shortening (DAMJANOVIC *et al.*, 2007). Therefore, a putative association between psychological stress and telomere accelerated attrition has been suggested.

Bipolar disorder (BD) is a mood disorder that affects 2% to 4% of the general population (MERIKANGAS *et al.*, 2007). It is categorized into two main subtypes. Type I BD is defined by the presence of at least one manic episode, independently of the occurrence of depressive espisodes. Type II BD is marked by the presence of hippomanic and depressive episodes (GOODWIN; JAMISON, 2007). BD is associated with poor quality of life (JUDD *et al.*, 2005), family disturbances (JUDD; AKISKAL, 2003) and mortality greater than the general population (ANGST *et al.*, 2012). Patients have more cardiovascular diseases which account for one third of their deaths (WESTMAN *et al.*, 2013). Other causes of death include somatic diseases, external conditions and suicide. Curiously, despite being more frequent than in the general population, suicide is not the main cause of death among BD patients (WESTMAN *et al.*, 2013). Therefore, studies have suggested higher health vulnerability within this clinical group. BD can be hypothesized as presenting cumulative and chronic stress, as mood episodes, drug abuse, and other stressors progressively occur (KAPCZINSKI *et al.*, 2008). An important issue is that Hypothalamic-pituitary-adrenal axis (HPA) dysfunction persists even during remission of symptoms, indicating the prolonged

consequences of stress response (VIETA; GASTO; MARTINEZ DE OSABA; *et al.*, 1997). Furthermore, patients with HPA dysfunction seem to be more vulnerable to relapse in bipolar patients (VIETA; GASTO; MARTINEZ DE OSABA; *et al.*, 1997). These findings highlight the importance of clarifying stress response dynamics to improve both clinical and psychiatric prognosis.

Within bipolar disorder evaluation of telomere length, although one study (MANSOUR *et al.*, 2011) did not find any differences between groups, there is some evidence of shorter telomere length in bipolar patients in contrast with controls (RIZZO *et al.*, 2013; SIMON *et al.*, 2006) and also of a greater proportion of short telomeres within the bipolar group (ELVSÅSHAGEN *et al.*, 2011). (ELVSÅSHAGEN *et al.*, 2011) suggested that the number of previous depressive episodes might be related to telomere attrition in bipolar disorder. Considering that only a few studies have investigated telomere attrition and even fewer have analyzed clinical features related to it, it becomes relevant to target these aspects in order to clarify stress dynamics and health vulnerability in BD.

In this study, the aim was to verify whether bipolar disorder and its clinical specificities are associated with telomere shortening. The clinical aspects considered were those reported to be involved in BD patient prognosis, including comorbidities and disorder subtype (DALTON *et al.*, 2003) (VIETA; GASTO; OTERO; *et al.*, 1997). It was hypothesized that these factors could provide additional stress, aggravating telomere shortening.

4.2 METHODS

4.2.1 Sample

The sample comprised 85 patients and 95 control subjects matched by age, gender and sex. All patients were diagnosed with bipolar disorder and classified by a senior psychiatrist

according to its subtype and the following comorbidities: Generalized Anxiety Disorder, Panic, Alcoholism, Drug Abuse, Eating and Obsessive Compulsive Disorder. The instrument used for classification was a structured interview - MINI Plus 5.0 (SHEEHAN *et al.*, 1998) which follows DSM-IV criteria for Axis I psychiatric disorders. In addition, data for the presence of Borderline Personality Disorder was collected. The control group was selected using the structured interview cited above (AMORIM, 2000; SHEEHAN *et al.*, 1998) and subjects were included if they had no past or present history of psychiatric disorders.

Procedures used in this study, including the interview and DNA analyses, were explained to all subjects, who provided informed consent. This study was approved by the local ethics committee (CAAE: 21185713.2.00005149) COEP.

4.2.2 Telomere length assay

Peripheral blood samples were collected in tubes containing EDTA, followed by DNA extraction with high salt method (LAHIRI; SCHNABEL, 1993). DNA was quantified using a NanoDrop Spectrophotometer Thermo Scientific, Nanodrop 200 model, and diluted to 75ng in 96 well plates.

Telomere length was measured using a relative quantification method that has been used and described before (CAWTHON, 2002). Briefly, two master mixes were prepared using the following primer pairs: for telomeres GGTTTTT-GAGGGTGAGGGTGAGGGTGAGGGTGAGGGT and TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA, for control gene 36B4 CAGCAAGTGGGAAGGTGTAATCC and CCCATTCTATCATCAACGGGTACAA. Each reaction was performed in triplicate for each sample and averaged for further calculations. For PCR reactions, PlatinumTaq (Invitrogen) was used and amplicon formation was monitored using SYBR-Green fluorescent dye (Invitrogen). All PCR reactions and fluorescence

measures were carried out in an ABI-7500 real-time PCR machine (ABI). Each reaction was performed in triplicate for each sample and averaged for further calculations.

For telomere length quantification, cycle thresholds (Ct) for each telomere and control gene 36B4 PCR reaction were calculated using the ABI software algorithm. The telomere/control gene 36B4 (T/C) ratio reflects the size of telomere for each sample. Considering the exponential kinetics of the PCR reaction, this ratio may be expressed as the following equation: $2^{-\Delta Ct}$, where $-\Delta Ct = -$ (Ct telomere - Ct control gene 36B4) of sample n. For group comparisons, $2^{-\Delta Ct}$ values for each sample were grouped and analyzed together. Experiments were performed at least twice.

4.2.3 Statistical analyses

Groups were paired for demographic variables. Age was the only continuous variable with normal distribution and Student's t-test was used to check for significant difference between means. For telomere length comparison (T/C ratio), the Mann-Whitney U test was used to test for significant difference between means. For categorical variables, x^2 test was performed.

Within the BD group telomere length and clinical features were compared after dividing the experimental group in categories, according to subtype of BD, comorbidities and presence of borderline personality disorder.

Later, telomere length was also categorized in long and short telomeres in order to investigate possible risk factors among those patients with short telomeres. Volunteers with telomere length lower than whole sample median were compounded Short telomere group, those with telomere higher than median were Long telomere category. It was conducted comparisons between patients and controls within each range, and effect size calculated.

4.3 RESULTS

4.3.1 Bipolar disorder is associated with reduced telomere lengths

To evaluate if bipolar disorder is associated with shorter telomeres, telomere length of patients and control subjects were measured after dividing the patient group in categories according to subtypes of disorder. Groups were matched for age, sex and educational level (Table 1). BD patients had shorter mean telomere length compared to control subjects - with a moderate effect size of 0.36, regardless of the disorder subtype (Table 2). It is noteworthy that there was no significant difference in telomere length between BD subtypes and they were pooled together for further analysis. Therefore, these data suggest that bipolar disorder is associated with reduced telomeres.

Table 4.1 Sample Characteristics

	Patients N= 85	Control N= 96	P value
Telomere length (Median)	296.48	412.74	**0.00**
Age (Mean +- SD Years)	39.46 (10.63)	38.33 (11.02)	0.48
Sex, Woman, n (%)	21 (24.7)	35 (36.5)	0.06
Educational Level			
Primary school, n (%)	17 (20)	26 (27. 4)	0.47
Secondary school, n (%)	39 (45.9)	42 (44.2)	
Undergraduation, n (%)	29 (34.1)	27 (28.4)	

Telomere length maintenance is a dynamic process that may suffer the influence of many variables present other comorbidities, it is possible that telomere shortening represents a secondary event. In order to evaluate whether some other specific feature of BD patients has

a major role in telomere shortening, the presence of clinical comorbidities and mean telomere length in the BD group was compared (Table 3). There was no specific clinical comorbidity associated with a greater reduction of telomeres. This suggests that bipolar disorder is directly associated with telomere shortening and that, at least in this sample, no other clinical aspect evaluated had a major impact over the phenotype.

Table 4.2 Comparisons per type of bipolar disorder

	Telomere length median	*P*- value (effect size – Rosenthal *r)*
Control	412.74	
BD I	295.06	**0.00**
BD II	309.08	
Control	412.74	**0.00**
BD I	295.06	**(-0.36)**
Control	412.74	**0.003**
BD II	309.08	**(-0.27)**
BD I	295.06	0.85
BDII	309.08	(-0.02)

Recently, a study has shown that a relative telomere length difference of 118.07 corresponds to approximately 278.9 base pairs difference (WOLKOWITZ *et al.*, 2011). This is comparable to seven years of aging (WOLKOWITZ *et al.*, 2011). Curiously, in the control group, age has a positive significant correlation with telomere length shortening (rho= - 0.27, p = 0,008) that is not mirrored in the BD group (rho= 0.60, p= 0.58). This suggests that bipolar disorder may mimic the effect of chronological age over telomere length.

Table 4.3 Comparisons according to comorbidity

	Present *n* (%) Median	Absent *n* (%) Median	*P*- value
Generalized Anxiety Disorder	32 (38.6) 294.57	51 (61.4) 297.55	0.60
Panic	20 (24.1) 276.75	63 (75.9) 323.49	0.22
Borderline	21 (25.3) 293.65	62 (74.7) 297.01	0.23
Alcoholism	25 (30.1) 291.40	58 (69.9) 300.78	0.92
Drugs	9 (11) 371.88	73 (89) 295.48	0.74
Eating Disorder	5 (6.2) 392.60	75 (93.8) 295.48	0.13
Obsessive Compulsive Disorder	6 (7.5) 384.57	74 (92.5) 294.57	0.83

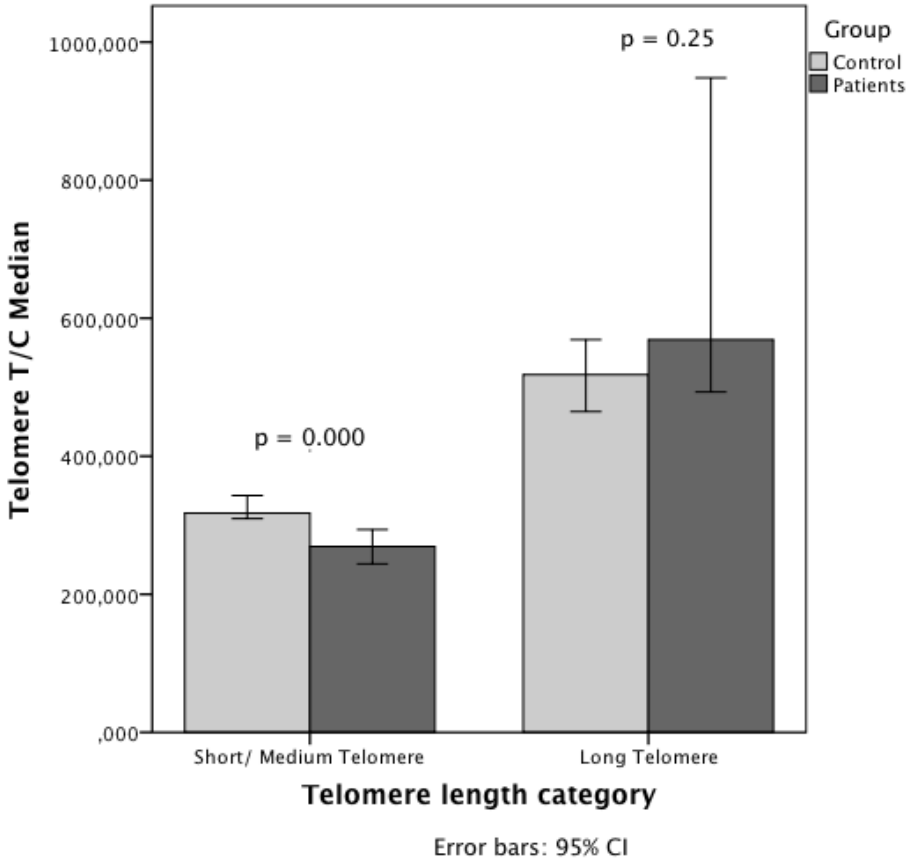
4.3.2 Bipolar disorder does not predict telomere length in a sample of long telomere subjects

Shorter telomeres were observed for BD patients when control and patient subjects were all taken together for comparison. However, it could be that this patient versus control difference was not homogenous for all telomere ranges, which could further suggest

protective or risk factors for patients in a specific category. Therefore, in order to investigate bipolar and control telomere difference across various ranges of telomere length, the whole sample was subdivided according to median and patients and controls were compared within each range.

In short telomere group, patients and controls differed significantly within this group, with a moderate effect size of 0.47. However, there was no difference when comparing subjects in the long telomere group (Figure 1). Furthermore, no differences were found between Short and Long telomere group regarding the following clinical variables: presence of panic disorder, obsessive compulsive disorder, generalized anxiety disorder, borderline personality disorder, type of bipolar disorder, number and type of past suicide attempt and number of comorbidities. Therefore, it was not possible to identify specific clinical factors that could explain the differences between patients and controls occurring only in the Short telomere group.

Figure 4.1. Comparison regarding telomere length category



4.4 DISCUSSION

In this study, the authors sought to discern any relation between bipolar disorder and telomere length, evaluating also some effects of comorbidity and BD subtype on telomere size. A reduction in telomeres of individuals suffering from BD was observed. In this sample, telomere size was not associated with any other clinical feature analyzed. Taken together these data suggest that bipolar disorder is directly associated with reduced telomere length, a feature similar to what is seen in aging. The set of comorbidities were selected because of their connection with poorer BP outcome (DALTON *et al.*, 2003; VIETA; GASTO; OTERO; *et al.*, 1997). It is hypothesized that they increase organismal stress and, therefore, patients presenting them may have shorter telomere length. However, in this study, those comorbidities were not associated with shorter telomeres. This is in accordance with other studies that did not observe this association (ELVSÅSHAGEN *et al.*, 2011; MARTINSSON *et al.*, 2013; RIZZO *et al.*, 2013; SIMON *et al.*, 2006)

In addition, some studies have shown that depression does not present any additional effect over accelerated telomere shortening, when associated with other conditions such as heart failure (HUZEN *et al.*, 2010) or aging (SANDERS *et al.*, 2012). These findings may indicate that some conditions may cover up the effects of others with regard to telomere attrition, which can be more sensitive than specific when reacting to life stress conditions (EPEL, ELISSA S. *et al.*, 2004). It can be supposed that BD is also disruptive and stressful enough, regardless of subtype, for any association with comorbidities not to have any additional effect on telomere size.

In addition to clinical aspects, age (SVENSON *et al.*, 2011), sex (BARRETT; RICHARDSON, 2011) and socioeconomic status (BATTY *et al.*, 2009) were reported to be associated with telomere length. It was observed that age determines telomere length in the control group but not in the BD group, suggesting that bipolar disorder may be a stressful condition that masks the age effect, breaking the linear relation of age and telomere shortening in patients.

It was investigated whether accelerated telomere shortening was homogenous throughout the BP patient sample. In the Long telomere group, patients and controls had similar telomere lengths while in the Short telomere group patients had significantly shorter telomeres. It was not found specific clinical vulnerability factors in Short telomere patients in contrast to Long telomere patients that could explain this patients X control difference occurring only in Short telomere group. Therefore, this result suggests that Long telomere patients may show protective factors that reduce vulnerability to telomere attrition. These features may not be shared with subjects in the Short Telomere Group. Further investigation should shed light on that finding, revealing new aspects of the modulation of the biology of neuropsychiatry disorders.

Studies have suggested that psychotropic drugs in schizophrenia and lithium treatment in BP are associated with protection against telomere shortening (SAVOLAINEN *et al.*, [S.d.]) (MARTINSSON *et al.*, 2013). In view of this, the medication data of a subsample of 30 BD patients was analyzed, which represents about 30% of patients. However, the results indicated no significant difference in telomere length when comparing users of the two most used mood stabilizers - Valproic acid versus Lithium - nor when comparing lithium users versus users of any other medication (data not shown). Since the patients came from a tertiary centre, polypharmacy was very frequent, which might have hidden any potential protective effect on the telomeres. It was not feasible to identify potential risk or protective factors. Apart from this limitation, some studies have shown BMI, genetic and environmental (BATTY *et al.*, 2009; VALDES *et al.*, 2005) interference on telomere length. However, these conditions could not be controlled in this study.

(ELVSÅSHAGEN *et al.*, 2011) suggest an association between number of previous depressive episodes and accelerated telomere shortening. Although this information was not collected from all patients we could gather data regarding number of previous manic and depressive episodes, as well as number and type of suicide attempt (absent, non-violent and violent) from 40 (47%) of the 85 patients included in this major study. None of these variables showed association with telomere length. Both hypotheses were tested as correlation analyses performed with continuous variables (data not shown).

In conclusion, this study has highlighted the heterogeneity of telomere dynamics in bipolar disorder. Understanding this aspect may be useful in understanding the disease in order to reduce bipolar patient's vulnerability to aging related diseases, thus enhancing their longevity and quality of life.

4.5 REFERENCES:

1. Blackburn EH. Telomere states and cell fates. Nature. 2000;408(6808):53–6.

2. Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. Febs Lett. 2005;579(4):859–62.

3. Chan SR, Blackburn EH. Telomeres and telomerase. Philos Trans R Soc Lond B Biol Sci. 2004;359(1441):109–22.

4. Babizhayev MA, Savel'yeva EL, Moskvina SN, Yegorov YE. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther. 2011;18(6):e209–e226.

5. Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. Aging Cell. 2011;10(6):913–21.

6. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. The Lancet. 2005;366(9486):662–4.

7. Zanni GR, Wick JY. Telomeres: unlocking the mystery of cell division and aging. Consult Pharm. 2011;26(2):78–90.

8. McEwen BS, Stellar E. Stress and the individual: mechanisms leading to disease. Arch Intern Med. 1993;153(18):2093.

9. McEwen BS. Mood disorders and allostatic load. Biol Psychiatry. 2003;54(3):200–7.

10. Wikgren M, Maripuu M, Karlsson T, Nordfjäll K, Bergdahl J, Hultdin J, et al. Short telomeres in depression and the general population are associated with a hypocortisolemic state. Biol Psychiatry. 15 de fevereiro de 2012;71(4):294–300.

11. Damjanovic AK, Yang Y, Glaser R, Kiecolt-Glaser JK, Nguyen H, Laskowski B, et al. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. J Immunol. 2007;179(6):4249–54.

12. Merikangas KR, Akiskal HS, Angst J, Greenberg PE, Hirschfeld R, Petukhova M, et al. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. Arch Gen Psychiatry. 2007;64(5):543.

13. Goodwin FK, Jamison KR. Manic-depressive illness: bipolar disorders and recurrent depression [Internet]. Oxford University Press; 2007 [citado 4 de dezembro de 2013]. Recuperado de: http://books.google.com.br/books?hl=pt-BR&lr=&id=hOHWEtEKEO4C&oi=fnd&pg=PR11&dq=goodwin+manic+depressive+illness&ots=H7UEv9DUTf&sig=cg57nHlrAtZ4FUnCY9cI45GRplQ

14. Judd LL, Akiskal HS, Schettler PJ, Endicott J, Leon AC, Solomon DA, et al. Psychosocial disability in the course of bipolar I and II disorders: a prospective, comparative, longitudinal study. Arch Gen Psychiatry. 2005;62(12):1322.

15. Judd LL, Akiskal HS. The prevalence and disability of bipolar spectrum disorders in the US population: re-analysis of the ECA database taking into account subthreshold cases. J Affect Disord. 2003;73(1):123–31.

16. Angst J, Hengartner MP, Gamma A, von Zerssen D, Angst F. Mortality of 403 patients with mood disorders 48 to 52 years after their psychiatric hospitalisation. Eur Arch Psychiatry Clin Neurosci. 2012;1–10.

17. Westman J, Hällgren J, Wahlbeck K, Erlinge D, Alfredsson L, Ösby U. Cardiovascular mortality in bipolar disorder: a population-based cohort study in Sweden. BMJ Open

[Internet]. 2013 [citado 4 de dezembro de 2013];3(4). Recuperado de: http://bmjopen.bmj.com/content/3/4/e002373.short

18. Kapczinski F, Vieta E, Andreazza AC, Frey BN, Gomes FA, Tramontina J, et al. Allostatic load in bipolar disorder: implications for pathophysiology and treatment. Neurosci Biobehav Rev. 2008;32(4):675–92.

19. Vieta E, Gasto C, Martinez de Osaba MJ, Nieto E, Canto TJ, Otero A, et al. Prediction of depressive relapse in remitted bipolar patients using corticotrophin-releasing hormone challenge test. Acta Psychiatr Scand. 1997;95(3):205–11.

20. Mansour H, Chowdari K, Fathi W, Elassy M, Ibrahim I, Wood J, et al. Does telomere length mediate associations between inbreeding and increased risk for bipolar I disorder and schizophrenia? Psychiatry Res. 30 de junho de 2011;188(1):129–32.

21. Rizzo LB, Do Prado CH, Grassi-Oliveira R, Wieck A, Correa BL, Teixeira AL, et al. Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. Bipolar Disord. 10 de setembro de 2013;

22. Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, et al. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol Psychiatry. 1 de setembro de 2006;60(5):432–5.

23. Elvsåshagen T, Vera E, Bøen E, Bratlie J, Andreassen OA, Josefsen D, et al. The load of short telomeres is increased and associated with lifetime number of depressive episodes in bipolar II disorder. J Affect Disord. dezembro de 2011;135(1-3):43–50.

24. Dalton EJ, Cate-Carter TD, Mundo E, Parikh SV, Kennedy JL. Suicide risk in bipolar patients: the role of co-morbid substance use disorders. Bipolar Disord. 2003;5(1):58–61.

25. Vieta E, Gasto C, Otero A, Nieto E, Vallejo J. Differential features between bipolar I and bipolar II disorder. Compr Psychiatry. 1997;38(2):98–101.

26. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59:22–33.

27. Amorim P. Mini International Neuropsychiatric Interview (MINI): validation of a short structured diagnostic psychiatric interview. Rev Bras Psiquiatr. 2000;22(3):106–15.

28. Lahiri DK, Schnabel B. DNA isolation by a rapid method from human blood samples: effects of MgCl2, EDTA, storage time, and temperature on DNA yield and quality. Biochem Genet. 1993;31(7-8):321–8.

29. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002;30(10):e47–e47.

30. Wolkowitz OM, Mellon SH, Epel ES, Lin J, Dhabhar FS, Su Y, et al. Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress-preliminary findings. PloS One. 2011;6(3):e17837.

31. Martinsson L, Wei Y, Xu D, Melas PA, Mathé AA, Schalling M, et al. Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres. Transl Psychiatry. 2013;3:e261.

32. Huzen J, van der Harst P, de Boer RA, Lesman-Leegte I, Voors AA, van Gilst WH, et al. Telomere length and psychological well-being in patients with chronic heart failure. Age Ageing. março de 2010;39(2):223–7.

33. Sanders JL, Fitzpatrick AL, Boudreau RM, Arnold AM, Aviv A, Kimura M, et al. Leukocyte telomere length is associated with noninvasively measured age-related disease: The Cardiovascular Health Study. J Gerontol A Biol Sci Med Sci. abril de 2012;67(4):409–16.

34. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, et al. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci U S A. 2004;101(49):17312–5.

35. Svenson U, Nordfjäll K, Baird D, Roger L, Osterman P, Hellenius M-L, et al. Blood cell telomere length is a dynamic feature. PloS One. 2011;6(6):e21485.

36. Batty GD, Wang Y, Brouilette SW, Shiels P, Packard C, Moore J, et al. Socioeconomic status and telomere length: the West of Scotland Coronary Prevention Study. J Epidemiol Community Health. 2009;63(10):839–41.

37. Savolainen K, Eriksson JG, Kananen L, Kajantie E, Hovatta I, Lahti M, et al. Combination of early life stress and traumatic experiences across the lifespan are associated with shorter leukocyte telomere length in later adulthood: the Helsinki Birth Cohort Study. Eur J Psychotraumatology. 3.

5. COMPARAÇÃO SEGUNDO TIPO DE TRATAMENTO E ASPECTOS CLÍNICOS ASSOCIADOS

Alguns estudos com transtornos psiquiátricos encontraram associações entre tratamento medicamentoso e efeitos protetivos em relação aos telômeros. (MARTINSSON *et al.*, [S.d.]) encontrou que o uso de lítio está associado a telômeros longos. Já os achados de (SAVOLAINEN *et al.*, [S.d.]) sugerem que uso de medicações psicotrópicas para o tratamento da esquizofrenia também pode oferecer ação protetiva estando associado a telômeros mais longos.

Com a finalidade de verificar possíveis viéses relacionados aos resultados demonstrados no tópico anterior, foram feitas análises com uma subamostra (N=29) de pacientes após a coleta dos dados sobre a mediação utilizada no período em que foi feita a coleta de sangue. As medicações (tabela 5.1) foram subdivididas segundo as classes correspondentes e as análises foram feiras através de teste de hipótese não paramétrico para comparação de grupos (teste de Mann-Whitney), tendo em vista que os dados a respeito do comprimento telomérico não apresentava distribuição normal. A primeira comparação foi realizada comparando-se o tratamento de lítio (n= 12), mesmo quando associado a outros medicamentos versus pacientes que não utilizavam lítio em seu tratamento (n=17). Os resultados não indicaram diferenças significativas entre o comprimento telomérico dos grupos (U= 81,00, p= 0,35)..

Tabela 5.1 Descrição do tipo de medicação utilizada segundo a classe medicamentosa

Classe	N	Porcentagem %
Antidepressivos	10	30, 30
Benzodiazepínico	15	51,72
Estabilizadores do humor	24	82,76
Antipsicóticos	14	48,28
Outros	4	13,79

Os dados consideraram uma subamostra total de 29 pacientes

Posteriormente, foi feita comparação entre os principais estabilizadores do humor utilizados sendo o Carbonato de Lítio (n= 12) versus Ácido Valpróico (n=11), a qual também não demonstrou resultados significativos (U= 48,00, p= 0,27).

Em uma terceira comparação considerando-se a medicação, os pacientes que apresentavam uso de antipsicóticos em contraposição com os demais não demonstraram qualquer tipo de associação entre comprimento telomérico e uso de antipsicóticos (U= 102,00; p= 0,89).

Os resultados apontam para ausência de efeitos da medicação sobre o comprimento telomérico. De toda forma, cabe a ressalva de que os pacientes inseridos neste estudos fazem uso de tratamento medicamentoso combinado, o que dificulta a caracterização de efeitos isolados, além de a subamostra utilizada ser reduzida (N=29).

Em relação aos aspectos clínicos que poderiam estar associados a um agravamento da experiência de estresse e, consequentemente, a um prejuízo ainda maior relacionado ao comprimento telomérico, (ELVSÅSHAGEN *et al.*, [S.d.]) indica que sujeitos com histórico de número de episódios depressivos maior ou igual a 20 apresentaram maior carga de telômeros curtos. Nos que diz respeito aos demais aspectos clínicos avaliados por estudos

prévios como presença do tipo ciclagem rápida de TAB (MARTINSSON *et al.*, [S.d.]), número de episódios hipomaníacos (ELVSÅSHAGEN *et al.*, [S.d.]), duração da doença (ELVSÅSHAGEN *et al.*, [S.d.]) ou associação de quadro de ansiedade ao TAB (SIMON *et al.*, [S.d.]), nenhum deles encontraram associações entre aspectos clínicos e encurtamento telomérico.

Diante da impossibilidade de coleta de dados de todos os sujeitos, neste estudo avaliamos aspectos clínicos específicos de uma subamostra de 29 pacientes. Foram realizadas análises de correlação entre o comprimento telomérico e número de episódios depressivos (*rho*=0,008; *p*=0,96), episódios maníacos prévios (*rho*=- 0,051; *p*=0,76), além de dados a respeito de histórico de tentativas de suicídio (não violenta, violenta, e ausente) - Tabela 5.2. Se o paciente apresentou pelo menos uma tentativa de suicídio violenta no passado, ele foi incluído na classe histórico de tentativas violentas.

Tabela 5.2 Comparações de comprimento telomérico de pacientes segundo o histórico de tentativa de suicídio

Histórico de tentativas de suicídio	Comprimento telomérico médio		
	Mediana	H	*p*
Ausênte	277,66		
Violenta	282,59	1,69	*p*=0,43
Não-violenta	379,64		

[a] Foi realizado o teste não-paramétrico de Kurskal-Wallis

Os resultados apontam para ausência de relação entre os aspectos clínicos avaliados e encurtamento de telômeros, achado que está em consonância com alguns dos estudos citados.

Os resultados sugerem que apesar de os pacientes apresentarem telômeros mais curtos em relação aos controles, as variáveis clínicas consideradas não representam medidas acuradas do efeito de estresse oxidativo associado ao transtorno, mecanismo considerado mediador entre condições psiquiátricas, encurtamento telomérico e vulnerabilidade a doenças associadas (doenças cardiovasculares, diabetes).

6. DISCUSSÃO

Os resultados indicam que pacientes, em comparação com controles, pareados por sexo, idade e escolaridade apresentam encurtamento telomérico mais acelerado. Este dado sugere que é possível que este grupo se apresente em vulnerabilidade quanto a fatores que predispõem tal aceleramento. Um destes fatores pode estar relacionado aos elevados níveis de estresse oxidativo, evidenciados em pacientes com transtornos do humor e especificamente, no transtorno bipolar (KAPCZINSKI *et al.*, [S.d.]). A presença de estresse oxidativo tem sido apontada como mecanismo envolvido no encurtamento telomérico, independente do problema do final da replicação (VON ZGLINICKI; PILGER; SITTE, 2000). Os resultados deste estudo corroboram a hipótese inicial que de o estresse oxidativo oriundo da psicopatologia pode gerar vulnerabilidade a nível de senescencia celular e, possivelmente, a maior predisposição a doenças relacionadas ao envelhecimento.

Outro fator de grande relevância para o agravamento do encurtamento telomérico é a presença de agentes proinflamatórios (HOHENSINNER; GORONZY; WEYAND, 2011) os quais se apresentam em abundância em pacientes com TAB (KIM *et al.*, 2007). Mecanismos inflamatórios estão associados a proliferação celular de leucócitos, consequentemente aumentando a perda de DNA telomérico através das sucessivas mitoses (HOHENSINNER; GORONZY; WEYAND, 2011). Os elevados níveis de estresse representam condições de simulações antigênicas que impõem demanda por aumento da atividade de células imunológicas (HOHENSINNER; GORONZY; WEYAND, 2011).

Apesar da maior vulnerabilidade do grupo clínico, não foi possível verificar nenhum fator clínico específico que contribuísse para esse aceleramento, sugerindo o rápido encurtamento como indício molecular de uma carga geral de estresse vivenciado. Este dado está de acordo com estudos que apontam para encurtamento telomérico em diversas condições, como em cuidadores (DAMJANOVIC *et al.*, [S.d.]), pacientes deprimidos

(WIKGREN *et al.*, [S.d.]), vitimas de (VALDES *et al.*, [S.d.]) adversidades na infância (KIECOLT-GLASER *et al.*, 2011), tabagistas (VALDES *et al.*, [S.d.]), e ainda pacientes sedentários referencia (VALDES *et al.*, [S.d.]).

No entanto, além de os dados indicarem que o encurtamento é uma medida sensível a diferentes tipos de estresse, o encurtamento aparentemente não ocorre de forma homogênea entre a amostra de sujeitos com TAB. Em concordância com esta heterogeneidade, outros estudos encontraram fatores de proteção e risco associados ao encurtamento telomérico (MARTINSSON *et al.*, [S.d.]; PUTERMAN *et al.*, 2013). Neste estudo, pacientes e controles da categoria de telômeros longos não apresentarem diferenças quanto ao comprimento telomérico. No entanto, não foi possível identificar potenciais fatores de risco que explicassem as diferenças entre pacientes e controles na categoria de telômeros curtos.

O quadro de TAB apresenta elevada comorbidade com doenças cardiovasculares, as quais são as principais causas de morte no mundo, sendo o risco de morte por doenças cardiovasculares duplicados na população com TAB tipo 1 em comparação com a população geral (ANGST *et al.*, 2013). Tendo em vista a associação entre comprimento telomérico, doenças cardiovasculares e câncer, principais patologias associadas à mortalidade global ("WHO | Reports", [S.d.]), é possível considerar o comprimento telomérico como um marcador de vulnerabilidade.

Os achados deste estudo apontam para a possibilidade de o encurtamento telomérico ser marcador de vulnerabilidade a doenças clínicas crônicas, e ainda ao estresse, sugerindo que além do problema do final da replicação, associados ao número de mitoses realizadas, outros processos podem afetar a velocidade de tal encurtamento.

7. CONCLUSÃO E PERSPECTIVAS FUTURAS

A disfuncionalidade que acomete pacientes com Transtorno Afetivo Bipolar envolve maior vulnerabilidade ao desemprego, inadimplência, baixa qualidade de vida, além de prejuízos cognitivos (JUDD *et al.*, [S.d.]; JUDD; AKISKAL, [S.d.]), e condições medicas gerais piores. Considerando o maior risco de doenças crônicas nesta população clínica e os elevados níveis de mortalidade e disfuncionalidade em pacientes com Transtorno Afetivo Bipolar (ANGST *et al.*, 2013), é possível considerar o transtorno como sendo psíquico e somático, conferindo maior vulnerabilidade geral à saúde do indivíduo.

O encurtamento telomérico em pacientes psiquiátricos, neste caso com TAB, é uma evidência da integração entre diversos aspectos relacionados à saúde, como imunologia, reatividade ao estresse, hábitos de vida, entre outros. A literatura mostra encurtamento de telômeros em diversas condições psiquiátricas e não-psiquiátricas. Um dos fatores subjacentes comuns a estas diversas patologias e condições é a exposição ao estresse e condições adversas.

O estresse, processo de adaptação do indivíduo ao longo da vida pressupõe a alostase, ativação de mecanismos mediadores do processo de restabelecimento da homeostase (STERLING; EYER, 1988). Um dos mediadores da alostase são respostas do sistema neuroendócrino, sistema nervoso autonômico e sistema imunológico, ativados em resposta a estressores ou fatores de vida diária. Essas respostas, em geral são benéficas (MCEWEN, 2003). No entanto, quando ocorrem disfunções através da sua ativação exacerbada, mudanças graduais vão ocorrendo neste organismo gerando um custo de adaptação, que é chamado de carga alostática (STERLING; EYER, 1988) .

Considerando que mecanismos pro-inflamatórios e estresse oxidativo sistêmico são mediadores da carga alostática e que o comprimento telomérico está associado a estes fatores (VON ZGLINICKI, [S.d.]) , é possível conceber o comprimento de telômeros, como uma,

mas não a única, medida de vulnerabilidade global do organismo, de modo que as condições psiquiátricas e clínicas estão intimamente ligadas

Assim, o fato de o encurtamento ocorrer em diversas patologias ou condições e a dificuldade de determinar fatores de risco específicos apontam para o comprimento telomérico como indicador do reflexo de uma série de sistemas afetados pela quebra na homeostase do organismo. A inespecificidade do achado indica integração entre eles e aponta para uma resposta do organismo como um todo a estas condições adversas e de estresse.

Diante da necessidade reduzir a vulnerabilidade e disfuncionalidade de pacientes com TAB e aqueles submetidos a condições adversas, pesquisas futuras devem continuar verificando estratégias que desacelerem o encurtamento de telômeros, como modificação de hábitos de vida (EPEL, ELISSA *et al.*, 2009), e que confiram correspondente redução da vulnerabilidade geral a esses sujeitos.

8. REFERÊNCIAS BIBLIOGRÁFICAS

Amorim, P. (2000). Mini International Neuropsychiatric Interview (MINI): validation of a short structured diagnostic psychiatric interview. *Revista Brasileira de Psiquiatria*, *22*(3), 106–115.

Angst, J., Hengartner, M. P., Gamma, A., von Zerssen, D., & Angst, F. (2012). Mortality of 403 patients with mood disorders 48 to 52 years after their psychiatric hospitalisation. *European archives of psychiatry and clinical neuroscience*, 1–10.

Association, A. P., & DSM-IV, A. P. A. T. F. on. (2000). *Diagnostic and statistical manual of mental disorders: DSM-IV-TR.* American Psychiatric Publishing, Inc.

Aubert, G., & Lansdorp, P. M. (2008). Telomeres and aging. *Physiological reviews*, *88*(2), 557–579.

Babizhayev, M. A., Savel'yeva, E. L., Moskvina, S. N., & Yegorov, Y. E. (2011). Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. *American journal of therapeutics*, *18*(6), e209–e226.

Barrett, E. L., & Richardson, D. S. (2011). Sex differences in telomeres and lifespan. *Aging cell*, *10*(6), 913–921.

Batty, G. D., Wang, Y., Brouilette, S. W., Shiels, P., Packard, C., Moore, J., … Ford, I. (2009). Socioeconomic status and telomere length: the West of Scotland Coronary Prevention Study. *Journal of epidemiology and community health*, *63*(10), 839–841.

Benes, F. M., Matzilevich, D., Burke, R. E., & Walsh, J. (2006). The expression of proapoptosis genes is increased in bipolar disorder, but not in schizophrenia. *Molecular psychiatry*, *11*(3), 241–251.

Blackburn, E. H. (2000). Telomere states and cell fates. *Nature*, *408*(6808), 53–56.

Blackburn, E. H. (2001). Switching and signaling at the telomere. *Cell*, *106*(6), 661–673.

Blackburn, E. H. (2005). Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *Febs Letters*, *579*(4), 859–862.

Blasco, M. A. (2007). Telomere length, stem cells and aging. *Nature chemical biology*, *3*(10), 640–649.

Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G., … de Geus, E. J. (2013). Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics*, *21*(10), 1163–1168.

Canela, A., Vera, E., Klatt, P., & Blasco, M. A. (2007). High-throughput telomere length quantification by FISH and its application to human population studies. *Proceedings of the National Academy of Sciences*, *104*(13), 5300.

Cavanagh, J. T. O., Van Beck, M., Muir, W., & Blackwood, D. H. R. (2002). Case—control study of neurocognitive function in euthymic patients with bipolar disorder: an association with mania. *The British Journal of Psychiatry*, *180*(4), 320–326.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic acids research*, *30*(10), e47–e47.

Chan, S. R., & Blackburn, E. H. (2004). Telomeres and telomerase. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1441), 109–122.

Dalton, E. J., Cate-Carter, T. D., Mundo, E., Parikh, S. V., & Kennedy, J. L. (2003). Suicide risk in bipolar patients: the role of co-morbid substance use disorders. *Bipolar Disorders*, *5*(1), 58–61.

Damjanovic, A. K., Yang, Y., Glaser, R., Kiecolt-Glaser, J. K., Nguyen, H., Laskowski, B., … Weng, N. (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *The Journal of Immunology*, *179*(6), 4249–4254.

Elvsåshagen, T., Vera, E., Bøen, E., Bratlie, J., Andreassen, O. A., Josefsen, D., … Boye, B. (2011). The load of short telomeres is increased and associated with lifetime number of depressive episodes in bipolar II disorder. *Journal of Affective Disorders*, *135*(1-3), 43–50. doi:10.1016/j.jad.2011.08.006

Epel, E., Daubenmier, J., Moskowitz, J. T., Folkman, S., & Blackburn, E. (2009). Can meditation slow rate of cellular aging? Cognitive stress, mindfulness, and telomeres. *Annals of the New York Academy of Sciences*, *1172*(1), 34–53.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., & Cawthon, R. M. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(49), 17312–17315.

Goodwin, F. K., & Jamison, K. R. (2007). *Manic-depressive illness: bipolar disorders and recurrent depression* (Vol. 1). Oxford University Press. Recuperado de

http://books.google.com.br/books?hl=pt-BR&lr=&id=hOHWEtEKEO4C&oi=fnd&pg=PR11&dq=goodwin+manic+depressive+illness&ots=H7UEv9DUTf&sig=cg57nHlrAtZ4FUnCY9cI45GRplQ

Harley, C. B., Futcher, A. B., & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts.

Hayflick, L. (1965). THE LIMITED IN ‘c/ITRO LIFETIME OF HUMAN DIPLOID CELL STRAINS1, 2. *Experimental cell research*, *37*, 614–636.

Hayflick, L., Moorhead, P. S., & others. (2006). The serial cultivation of human diploid cell strains. *Experimental cell research*, *25*(3), 585–621.

Hohensinner, P. J., Goronzy, J. J., & Weyand, C. M. (2011). Telomere dysfunction, autoimmunity and aging. *Aging and disease*, *2*(6), 524.

Huzen, J., van der Harst, P., de Boer, R. A., Lesman-Leegte, I., Voors, A. A., van Gilst, W. H., … van Veldhuisen, D. J. (2010). Telomere length and psychological well-being in patients with chronic heart failure. *Age and Ageing*, *39*(2), 223–227. doi:10.1093/ageing/afp256

Iwamoto, K., Bundo, M., & Kato, T. (2005). Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Human Molecular Genetics*, *14*(2), 241–253.

Jiang, H., Schiffer, E., Song, Z., Wang, J., Zürbig, P., Thedieck, K., … others. (2008). Proteins induced by telomere dysfunction and DNA damage represent biomarkers of human aging and disease. *Proceedings of the National Academy of Sciences*, *105*(32), 11299.

Judd, L. L., & Akiskal, H. S. (2003). The prevalence and disability of bipolar spectrum disorders in the US population: re-analysis of the ECA database taking into account subthreshold cases. *Journal of affective disorders*, *73*(1), 123–131.

Judd, L. L., Akiskal, H. S., Schettler, P. J., Endicott, J., Leon, A. C., Solomon, D. A., … Keller, M. B. (2005). Psychosocial disability in the course of bipolar I and II disorders: a prospective, comparative, longitudinal study. *Archives of General Psychiatry*, *62*(12), 1322.

Kapczinski, F., Vieta, E., Andreazza, A. C., Frey, B. N., Gomes, F. A., Tramontina, J., … Post, R. M. (2008a). Allostatic load in bipolar disorder: implications for pathophysiology and treatment. *Neuroscience & Biobehavioral Reviews*, *32*(4), 675–692.

Kapczinski, F., Vieta, E., Andreazza, A. C., Frey, B. N., Gomes, F. A., Tramontina, J., … Post, R. M. (2008b). Allostatic load in bipolar disorder: implications for pathophysiology and treatment. *Neuroscience & Biobehavioral Reviews*, *32*(4), 675–692.

Kiecolt-Glaser, J. K., Gouin, J.-P., Weng, N., Malarkey, W. B., Beversdorf, D. Q., & Glaser, R. (2011). Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. *Psychosomatic medicine*, *73*(1), 16–22.

Kim, Y.-K., Jung, H.-G., Myint, A.-M., Kim, H., & Park, S.-H. (2007). Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. *Journal of affective disorders*, *104*(1), 91–95.

Korte, S. M., Koolhaas, J. M., Wingfield, J. C., & McEwen, B. S. (2005). The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neuroscience & Biobehavioral Reviews*, *29*(1), 3–38.

Lahiri, D. K., & Schnabel, B. (1993). DNA isolation by a rapid method from human blood samples: effects of MgCl2, EDTA, storage time, and temperature on DNA yield and quality. *Biochemical genetics*, *31*(7-8), 321–328.

MacDonald, M. L., Naydenov, A., Chu, M., Matzilevich, D., & Konradi, C. (2006). Decrease in creatine kinase messenger RNA expression in the hippocampus and dorsolateral prefrontal cortex in bipolar disorder. *Bipolar disorders*, *8*(3), 255–264.

Mansour, H., Chowdari, K., Fathi, W., Elassy, M., Ibrahim, I., Wood, J., … Nimgaonkar, V. L. (2011). Does telomere length mediate associations between inbreeding and increased risk for bipolar I disorder and schizophrenia? *Psychiatry Research*, *188*(1), 129–132. doi:10.1016/j.psychres.2011.01.010

Martinsson, L., Wei, Y., Xu, D., Melas, P. A., Mathé, A. A., Schalling, M., … Backlund, L. (2013). Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres. *Translational Psychiatry*, *3*, e261. doi:10.1038/tp.2013.37

McEwen, B. S. (2003). Mood disorders and allostatic load. *Biological psychiatry*, *54*(3), 200–207.

McEwen, B. S., & Stellar, E. (1993). Stress and the individual: mechanisms leading to disease. *Archives of internal medicine*, *153*(18), 2093.

Merikangas, K. R., Akiskal, H. S., Angst, J., Greenberg, P. E., Hirschfeld, R., Petukhova, M., & Kessler, R. C. (2007). Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. *Archives of general psychiatry*, *64*(5), 543.

Petersen, S., Saretzki, G., & Zglinicki, T. von. (1998). Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Experimental cell research*, *239*(1), 152–160.

Puterman, E., Epel, E. S., Lin, J., Blackburn, E. H., Gross, J. J., Whooley, M. A., & Cohen, B. E. (2013). Multisystem resiliency moderates the major depression–Telomere length association: Findings from the Heart and Soul Study. *Brain, behavior, and immunity*, *33*, 65–73.

Rizzo, L. B., Do Prado, C. H., Grassi-Oliveira, R., Wieck, A., Correa, B. L., Teixeira, A. L., & Bauer, M. E. (2013). Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. *Bipolar Disorders*. doi:10.1111/bdi.12121

Rudolph, K. L. (2010). *Telomeres and Telomerase in Ageing, Disease, and Cancer*. Springer. Recuperado de http://link.springer.com/content/pdf/10.1007/978-3-540-73709-4.pdf

Sanders, J. L., Fitzpatrick, A. L., Boudreau, R. M., Arnold, A. M., Aviv, A., Kimura, M., … Newman, A. B. (2012). Leukocyte telomere length is associated with noninvasively measured age-related disease: The Cardiovascular Health Study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *67*(4), 409–416. doi:10.1093/gerona/glr173

Savolainen, K., Eriksson, J. G., Kananen, L., Kajantie, E., Hovatta, I., Lahti, M., … Räikkönen, K. ([s.d.]). Combination of early life stress and traumatic experiences across the lifespan are associated with shorter leukocyte telomere length in later adulthood: the Helsinki Birth Cohort Study. *European Journal of Psychotraumatology*, *3*.

Shay, J. W., & Wright, W. E. (2000). Hayflick, his limit, and cellular ageing. *Nature reviews Molecular cell biology*, *1*(1), 72–76.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of clinical psychiatry*, *59*, 22–33.

Simon, N. M., Smoller, J. W., McNamara, K. L., Maser, R. S., Zalta, A. K., Pollack, M. H., … Wong, K.-K. (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biological Psychiatry*, *60*(5), 432–435. doi:10.1016/j.biopsych.2006.02.004

Sterling, P., & Eyer, J. (1988). Allostasis: a new paradigm to explain arousal pathology. Recuperado de http://psycnet.apa.org/psycinfo/1988-98352-034

Sun, X., Wang, J.-F., Tseng, M., & Young, L. T. (2006). Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *Journal of Psychiatry and Neuroscience*, *31*(3), 189.

Svenson, U., Nordfjäll, K., Baird, D., Roger, L., Osterman, P., Hellenius, M.-L., & Roos, G. (2011). Blood cell telomere length is a dynamic feature. *PloS one*, *6*(6), e21485.

Tan, Z. (1999). Intramitotic and intraclonal variation in proliferative potential of human diploid cells: explained by telomere shortening. *Journal of theoretical biology*, *198*(2), 259–268.

Valdes, A. M., Andrew, T., Gardner, J. P., Kimura, M., Oelsner, E., Cherkas, L. F., … Spector, T. D. (2005). Obesity, cigarette smoking, and telomere length in women. *The Lancet*, *366*(9486), 662–664.

Vieta, E., Gasto, C., Martinez de Osaba, M. J., Nieto, E., Canto, T. J., Otero, A., & Vallejo, J. (1997). Prediction of depressive relapse in remitted bipolar patients using corticotrophin-releasing hormone challenge test. *Acta Psychiatrica Scandinavica*, *95*(3), 205–211.

Vieta, E., Gasto, C., Otero, A., Nieto, E., & Vallejo, J. (1997). Differential features between bipolar I and bipolar II disorder. *Comprehensive psychiatry*, *38*(2), 98–101.

Von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in biochemical sciences*, *27*(7), 339–344.

Von Zglinicki, T., Pilger, R., & Sitte, N. (2000). Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radical Biology and Medicine*, *28*(1), 64–74.

Westman, J., Hällgren, J., Wahlbeck, K., Erlinge, D., Alfredsson, L., & Ösby, U. (2013). Cardiovascular mortality in bipolar disorder: a population-based cohort study in Sweden. *BMJ open*, *3*(4). Recuperado de http://bmjopen.bmj.com/content/3/4/e002373.short

WHO | Reports. ([s.d.]). *WHO*. Recuperado 18 de fevereiro de 2014, de http://www.who.int/gho/publications/en/

Wikgren, M., Maripuu, M., Karlsson, T., Nordfjäll, K., Bergdahl, J., Hultdin, J., … Norrback, K.-F. (2012). Short telomeres in depression and the general population are associated with a hypocortisolemic state. *Biological Psychiatry*, *71*(4), 294–300. doi:10.1016/j.biopsych.2011.09.015

Wolkowitz, O. M., Mellon, S. H., Epel, E. S., Lin, J., Dhabhar, F. S., Su, Y., … others. (2011). Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress-preliminary findings. *PloS one*, *6*(3), e17837.

Wong, K. K., Maser, R. S., Bachoo, R. M., Menon, J., Carrasco, D. R., Gu, Y., … others. (2003). Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. *Nature*, *421*(6923), 643–648.

Zanni, G. R., & Wick, J. Y. (2011). Telomeres: unlocking the mystery of cell division and aging. *The Consultant Pharmacist*, *26*(2), 78–90.

9. ANEXO 1- ATA DE DEFESA

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA MOLECULAR

ATA DA DEFESA DA DISSERTAÇÃO DA ALUNA

ISABELA MARIA MAGALHÃES LIMA - 2012737450

Realizou-se, no dia 08 de maio de 2014, às 13:30 horas, Faculdade de Medicina, térreo, sala 062, da Universidade Federal de Minas Gerais, a defesa de dissertação, intitulada *Verificação de comprimento telomérico em pacientes com Transtorno Afetivo Bipolar*, apresentada por ISABELA MARIA MAGALHÃES LIMA, número de registro 2012737450, graduada no curso de PSICOLOGIA, como requisito parcial para a obtenção do grau de Mestre em MEDICINA MOLECULAR, à seguinte Comissão Examinadora: Prof. Debora Marques de Miranda - Orientador (UFMG), Prof. Leandro Fernandes Malloy Diniz (UFMG) - coorientador, Prof. Fernando Silva Neves (UFMG), Prof. Renan Pedra de Souza (UFMG).

A Comissão considerou a dissertação:

(X) Aprovada

( ) Reprovada

Finalizados os trabalhos, lavrei a presente ata que, lida e aprovada, vai assinada por mim e pelos membros da Comissão.
Belo Horizonte, 08 de maio de 2014.

Prof(a). Debora Marques de Miranda (Doutora)

Prof. Leandro Fernandes Malloy Diniz (Doutor)

Prof. Fernando Silva Neves (Doutor)

Prof. Renan Pedra de Souza (Doutor)

10. ANEXO 2- FOLHA DE APROVAÇÃO

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA MOLECULAR

FOLHA DE APROVAÇÃO

Verificação de comprimento telomérico em pacientes com Transtorno Afetivo Bipolar

ISABELA MARIA MAGALHÃES LIMA

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em MEDICINA MOLECULAR, como requisito para obtenção do grau de Mestre em MEDICINA MOLECULAR, área de concentração MEDICINA MOLECULAR.

Aprovada em 08 de maio de 2014, pela banca constituída pelos membros:

Prof(a). Débora Marques de Miranda - Orientador
UFMG

Prof. Leandro Fernandes Malloy Diniz
UFMG

Prof. Fernando Silva Neves
UFMG

Prof. Renan Pedra de Souza
UFMG

Belo Horizonte, 8 de maio de 2014.