UNIVERSIDADE FEDERAL FLUMINENSE

FACULDADE DE ODONTOLOGIA

POLIMORFISMOS NO GENE *ESRRB* ESTÃO ASSOCIADOS COM AS DESORDENS TEMPOROMANDIBULARES E LESÃO DO MANGUITO ROTADOR

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Área de Concentração: Clínica Odontológica

Orientador: Profa. Dra. Priscila Ladeira Casado
Co-orientador: Prof. Dr. José Mauro Granjeiro

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RESUMO

Bonato LL. Polimorfismos no gene *ESRRB* estão associados com as Desordens Temporomandibulares e Lesão do Manguito Rotador [dissertação]. Niterói: Universidade Federal Fluminense, Faculdade de Odontologia; 2014.

Os diversos sintomas físicos presentes nas Desordens Temporomandibulares (DTM) estão associados frequentemente à presença de doenças comórbidas. A dor no ombro é uma das principais queixas dos pacientes com DTM. O objetivo deste estudo foi investigar a base biomecânica da associação entre DTM e lesão do manguito rotador (LMR). Neste estudo transversal, foram avaliados fatores etiológicos comuns à DTM e LMR, a fim de elucidar a interação DTM/LMR. A avaliação das estruturas orofaciais (RDC-Eixo I) e da região do ombro foram realizadas em 108 indivíduos. O grupo controle consistiu de 30 integrantes sem queixas de dor. Setenta e oito indivíduos foram divididos em 3 grupos: com LMR (sem DTM, n=16), com DTM (sem LMR, n=13) e com ambas as lesões (com DTM+LMR, n=49). Um total de 8 polimorfismos do gene *ESRRB* envolvido na via do estrogênio foram investigados. Os níveis de estradiol foram mensurados por imunoensaio quimioluminescente. A atividade muscular dos músculos cervicais e faciais foi avaliada através de eletromiografia de superfície (EMG). A significância das variáveis nominais e contínuas foi analisada através dos testes do qui-quadrado, T-Student / Mann-Whitney, respectivamente Valores de $p<0.05$ foram considerados significantes. A regressão logística multivariada explorou múltiplas variáveis simultaneamente. Indivíduos com DTM apresentaram 7 vezes mais chances de desenvolver LMR (OR 7.0; 95% CI, 2.7-18.4). O risco de desenvolver ambas as lesões foi 6 vezes maior em caucasianos (OR, 5,9 ; IC 95 % , 1,9-18,5). Os genótipos TT rs1676303 (p=0.02) e GG rs6574293 (p=0.04) foram associadas à LMR. Indivíduos com ambas as lesões apresentaram maior frequência dos genótipos polimórficos para o rs4903399 (p = 0,02) e rs10132091 (p = 0,02). Os haplótipos CTTCTTAG (p = 0,01) e CCTCTCAG (p = 0,01) foram associados com DTM+LMR. A regressão logística multivariada afirmou caucasianos (p = 0,001) como um fator de risco para DTM+LMR. Os níveis de estradiol foram semelhantes entre os grupos. Os músculos masseter e temporal anterior apresentaram menor atividade

em pacientes com as duas doenças em posição de repouso (p=0,03 / p=0,02, respectivamente) e na posição de máxima intercuspidação habitual (p= 0,01 / p=0,03, respectivamente). Os músculos esternocleidomastóideo e trapézio apresentaram menor atividade em indivíduos com DTM+LMR (p = 0,03). Este presente trabalho sugere ser a DTM um fator de risco para a LMR. Etnia branca, haplótipos do gene *ESRRB* e baixa atividade eletromiográfica foram identificadas como características biomecânicas comuns nos indivíduos com ambas as lesões.

Palavras-chave: Síndrome da Disfunção da Articulação Temporomandibular , Artralgia, Manguito Rotador, Estrogênio.

ABSTRACT

Bonato LL. *ESRRB* polymorphisms are associated with comorbid Temporomandibular Disorders and Rotator Cuff disease [dissertation]. Niterói: Universidade Federal Fluminense, Faculdade de Odontologia; 2014.

Temporomadibular disorder's (TMD) physical symptoms are associated with comorbid disorders. Shoulder pain is one of the main complaints of TMD patients. The purpose of this study was to investigate the association between TMD and rotator cuff disease (RCD) comorbid symptoms and its biomechanical basis. In this cross-sectional study, we assessed for common etiology factors of TMD and RCD in order to elucidate the TMD/RCD interaction. Orofacial (RDC-Axis I) and shoulder examinations were performed in 108 subjects. The control group consisted of 30 subjects without pain. Seventy-eight committed subjects were divided into the following groups: RCD subjects (TMD free, n=16), TMD (RCD free, n=13) and TMD/RCD affected (with RCD + TMD, n=49). A total of 8 SNPs within the *ESRRB* gene involved in the estrogen pathway were investigated. Estradiol levels were measured by chemiluminescent immunoassay. The head and cervical muscle activity was recorded with surface electromyography (EMG). Significance of nominal and continuous variables was assessed by the chi-square test and Student t-/ Mann-Whitney tests. Values of $p<0.05$ were considered significant. Multivariate logistic regression permitted the exploration of covariates simultaneously. Subjects with TMD were seven times more susceptible to RCD (OR 7.0; 95% CI, 2.7-18.4) than subjects with healthy TMJ. The risk associated with having both diseases was 6 times higher in Whites (OR, 5.9; 95% CI, 1.9-18.5) than in the control. The rs1676303 TT genotype (p=0.02) and rs6574293 GG genotype (p=0.04) are associated with RCD. TMD/RCD affected subjects showed higher association with rs4903399 (p=0.02) and rs10132091 (p=0.02). Haplotype CTTCTTAG (p=0.01) and CCTCTCAG (p=0.01) were associated with TMD/RCD. Multivariate logistic regression affirmed being White (p=0.001) as a risk factor for TMD/RCD. Estradiol levels were similar among groups. Masseter and Temporalis muscles showed lower activity in patients with both diseases in rest position (p=0.03/ p=0.02, respectively) and in the maximum clenching effort (p=0.01/ p=0.03, respectively). Sternocleidomastoid and trapezius

showed lower activity in TMD/RCD affected subjects (p=0.03). Our work supported TMD as a risk factor for RCD. White ethnicity, *ESRRB* haplotypes, and low muscle surface EMG activity were identified as common biolmechanical characteristics in subjects with both diseases.

Keywords: Temporomandibular Joint Dysfunction Syndrome, Arthralgia, Rotator Cuff, Estrogens .

1 - INTRODUÇÃO

As Desordens Temporomandibulares (DTM), caracterizadas por alterações nos músculos mastigatórios e/ou na articulação Temporomandibular (ATM),[5,15] vêm apresentando atualmente aumento significativo e constante de sua prevalência na população mundial.[5,6] No entanto, sua complexa etiopatogenia e variabilidade de sintomas existentes, tornam difícil a adoção de protocolos diagnósticos e terapêuticos padronizados.[35,45] Adicionalmente, em pacientes com dores crônicas, como muitos dos portadores de DTM, existe a sobreposição de sintomas físicos entre a desordem e outras comorbidades, acarretando dores em diversos músculos e articulações, problemas cognitivos, e mudanças no padrão e qualidade do sono.[41] No passado, este perfil de paciente multiqueixoso, teve seu diagnóstico frequentemente subestimado, sendo atribuídos os quadros álgicos difusos puramente a fatores psicológicos.[35,41]

Atualmente, com a evolução das pesquisas, muito é estudado a cerca da "Síndrome da Sensibilização Central",[41] desconsiderando-se frequentemente outras características importantes, tais como presença de substâncias pró-inflamatórias,[62,67] traumas/lesões, moduladores presentes principalmente em DTM articulares, e aspectos genéticos.[67] Estes fatores podem estar relacionados a alterações em outras articulações do corpo, e com isso, justificar o desenvolvimento de dores e até mesmo lesões articulares simultâneas. Acredita-se ainda que cada um desses fatores seja modulado por numerosos processos biológicos, e estes, influenciados por diversos genes.[34,67]

Apesar da escassez de testes diagnósticos para avaliação de quadros álgicos difusos,[34,41,45] é reconhecido que dores sistêmicas são capazes de potencializar a dor orofacial em pacientes com DTM,[34,45,46,47,48] assim como as DTM são capazes de influenciar outras dores e ou lesões existentes. Dentre estas, pode-se ressaltar as dores na região dos ombros, as quais se apresentam como uma das principais queixas que acompanham o portador da Desordem Temporomandibular.[34,50]

Dentre as mais prevalentes causas de dores na região dos ombros por sua vez, estão as decorrentes da Lesão do Manguito Rotador (LMR).[52] Esta lesão é caracterizada por queixas dolorosas locais inicialmente,[22,23,27] mas que em muitos indivíduos, com o aumento da intensidade dolorosa, torna-se generalizada,

dificultando a localização precisa da lesão original.[18,22,33] Atualmente, acredita-se que a LMR acometa cerca de metade da população acima dos 50 anos de idade, sendo a intensidade da dor considerada maior no sexo feminino, e localizada principalmente entre os dermatomos C5 e C6.[22,33,44]

O manguito rotador é uma estrutura composta pelos músculos supra-espinhal, infra-espinhal, redondo menor e subescapular[44]. A etiologia específica relacionada a injúrias em sua estrutura, apesar de ainda não estar totalmente elucidada, é reflexo da combinação de fatores intrínsecos e extrínsecos.[52,59] Dentre os mesmos, pode-se citar: tendinopatias (parciais ou totais); alterações vasculares, bursite subaacromial,[25,26,56] degeneração intrínseca, fatores genéticos e síndrome do impacto.[18,27,52,55,57]

Na tentativa de compreender o desenvolvimento de dores conjuntas na região orofacial e ombros, pesquisas exploram a interação do complexo cabeça-pescoço-ombros. [8,53,61] Alterações na posição da cabeça podem influenciar quadros de DTM, ocasionando aumento da pressão sobre os músculos cervicais posteriores e da atividade muscular mastigatória, que por sua vez provoca hipercontração muscular, espasmos, alterações no posicionamento da escápula e consequentes dores nas regiões do pescoço e ombros.[8] Reiterando esta teoria, exercícios fisioterapêuticos são frequentemente recomendados no tratamento de indivíduos com LMR e/ou DTM, objetivando-se obter estabilidade articular, manutenção da postura, aumento da força e resistência, e, principalmente, indução da co-contração de músculos relacionados a ambas as articulações. Esta atividade é capaz de provocar altas respostas proprioceptivas e estimular receptores aferentes em torno das mesmas.[14,15]

Dentre outros aspectos que podem também influenciar em tal relação álgica, estão: a convergência neuronal de informações sensoriais a partir dos três primeiros nervos espinhais cervicais, com aferentes do trigêmeo;[5,34,35] sensibilização central promovida e mantida pela atividade neural entre as regiões-chave do cérebro envolvidas no processamento da dor crônica;[41,51] e fatores genéticos, que vem ganhando força devido à apresentação heterogênea de ambas as lesões.[67]

Dentre os estudos genéticos, são relatadas associações entre polimorfismos em diversos genes (genes relacionados à catechol-o-methyltransferase e dor crônica[48], *ANKH* e subtipos da desordem[30], enzimas da via metabólica do folato e estresse oxidativo inflamatório[2]) e diferentes incidências da DTM. Assim como foi

demonstrada associação entre LMR e degeneração tendínea (polimorfismos em genes *FGF3*, *FGF10*, *FGFR1*, *ESRRB*, *DEFB1*). [52] No entanto, até o momento, não existem estudos analisando aspectos genéticos relacionando simultaneamente as duas lesões.

Baseando-se no fato de que a estabilidade de tais articulações é diretamente influenciada por hormônios locais e sistêmicos, que o estrogênio influencia no desenvolvimento de quadros de osteoartrites,[21,35] mecanismos sensoriais,[39] inflamação e hipersensibilidade,[35] além de contribuir no desenvolvimento de diversas condições crônicas de dor,[10] e que os receptores de estrógeno (ERs) estão presentes na maioria - se não em todos - os tecidos, tornando as ações pleiotrópicas do mesmo extremamente importantes[21,31], acreditamos, que polimorfismos genéticos na via do estrógeno, possam estar relacionados à suscetibilidade dos indivíduos desenvolverem LMR e DTM.

Levando-se em conta que a DTM e a LMR são doenças multifatoriais comuns, o objetivo deste estudo foi investigar a associação entre os sintomas comórbidos da DTM e da LMR e sua base biomecânica, uma vez que o maior conhecimento sobre a associação destas doenças pode favorecer o diagnóstico e auxiliar no estabelecimento de protocolos terapêuticos mais eficazes

2 - METODOLOGIA

Este estudo transversal foi conduzido de acordo com as recomendações do Comitê de Ética em Pesquisa do Instituto Nacional de Traumatologia e Ortopedia (número do registro CAAE: 07723312.0.0000.5273), sendo o termo de consentimento livre e esclarecido obtido de cada participante. Cento e oito indivíduos, de ambos os sexos, foram selecionados aleatoriamente a partir de um mutirão de atendimentos no ambulatório deste mesmo hospital durante um ano. Os indivíduos responderam, inicialmente, a uma anamnese criteriosa sobre a história médica e odontológica pregressa. Estes, foram submetidos a consultas de rotina no Centro de Atendimento Especializado de Ombro e Cotovelo, a fim de avaliar o ombro e as condições da ATM. Os critérios de inclusão foram: ser brasileiro, possuir idade maior que 45 anos, e não ter sido submetido a cirurgia ou ter história de neoplasia nas regiões ATM/ombro. Pacientes com história de trauma, bursite, artrite reumatóide, doenças auto-imunes, gravidez, uso crônico de corticoides sistêmicos e aqueles diagnosticados com hipermobilidade articular sistêmica foram excluídos. O grupo controle foi composto por 30 indivíduos brasileiros, sem relato de dor, e sem sinais ou sintomas de DTM e LMR. Aqueles diagnosticados como tendo a LMR e/ou DTM foram divididos em três grupos: indivíduos com LMR (sem DTM, n=16), aqueles com DTM (sem LMR, n=13), e os que possuíam ambas as lesões (com LMR + DTM, n=49).

2.1 Diagnóstico das Desordens Temporomandibulares

Todos os participantes foram submetidos a exame clínico pelo mesmo dentista (autora L.L.B.) e o diagnóstico realizado de acordo com os Critérios de Diagnóstico para Pesquisa das Desordens Temporomandibulares (RDC/DTM) Eixo I[17], o qual permite que o diagnóstico seja dividido em três grupos: I – Diagnósticos musculares, II – Deslocamento de disco e III - Artralgia, artrite, artrose. O RDC/DTM foi utilizado utilizando técnicas validadas, incluindo a palpação em 20 sítios musculares bilateralmente[17]. O auto-relato de dores e/ou comprometimento funcional da região orofacial também foi considerado na avaliação.

Outras características clínicas foram observadas, como presença de: dores de ouvido, odontalgias, sensação de queimação na boca, limitação de abertura bucal e presença de ruídos articulares na ATM (estalidos ou creptações), bem como foi realizado o diagnóstico do bruxismo.[5] Do total, 62 indivíduos foram diagnosticados com a DTM.

2.2 Diagnóstico da Lesão do Manguito Rotador

Sessenta e cinco pacientes foram diagnosticados com LMR. O diagnóstico foi baseado no protocolo de Motta et al.[52], sendo realizado um exame clínico minucioso e utilizados exames de imagens (radiografia e ressonância magnética) do ombro envolvido. Indivíduos sem LMR deveriam ter ausência de dor no ombro, resultado negativo para o teste da síndrome do impacto[56] e ausência de tendinopatia. Todas as avaliações clínicas foram realizadas exclusivamente por um dos autores (M.V.A.) no Centro de Atenção Especializada.

Todos os registros do ortopedista que realizou o diagnóstico da LMR e do dentista que realizou o exame das estruturas orofaciais eram independentes uns dos outros.

2.3 Genotipagem do Receptor Relacionado ao Estrogênio tipo *β* (ESRRB)

O DNA genômico foi obtido a partir de amostras de saliva de todos os participantes, como anteriormente descrito.[36] A concentração e a pureza do DNA foram analisadas através da utilização do espectrofotômetro NanoDrop® (Thermo Scientific , Wilmington, DE, EUA). Todas as amostras deveriam apresentar a razão $A_{260\ nm}/A_{280\ nm}$ maiores do que 1,9.

Oito polimorfismos de um só nucleotídeo (SNPs) no gene *ESRRB* foram selecionados, considerando-se as relações de desequilíbrio de ligação e estrutura dos genes. Estes SNPs foram previamente identificados e incluídos na base de dados do Centro Nacional de Informações sobre Biotecnologia (http://www.ncbi.nlm.nih.gov/SNP/) devendo a menor frequência alélica ser >0,12. Todos os procedimentos seguiram as recomendações do STREGA[40].

A Análise de Endpoint de reações em cadeia da polimerase foi realizada utilizando-se o método de TaqMan (Applied Biosystems, Foster City CA, EUA),

utilizando um total de 1,5 mL/reação para a genotipagem dos marcadores selecionados em um termociclador PTC- 225 Tetrad (Peltier Termociclador, Bio-Rad Ciências da Vida, Hemel Hempstead, Hertfordshire, Reino Unido). Polimorfismos no gene ESRRB também foram analisados em combinação como haplótipos.

2.4 Nível do estradiol

Estrogênios endógenos são um grupo de compostos esteróides, que incluem o 17b-estradiol, a estrona e o estriol. Devido ao fato de o 17b-estradiol ser o composto mais bioativo dentre os três tipos16, optamos por avaliar a concentração de estradiol no soro.

Uma amostra de sangue de 5ml foi retirada de 41 indivíduos do grupo total da amostra (8 controles, 9 com LMR, 4 com DTM, e 20 com ambas as lesões) após 8 horas de jejum durante a noite. O sangue foi centrifugado, e, em seguida, as amostras de soro foram armazenadas a –20°C. A fim de excluir a influência de variáveis de hormônios nos níveis de estradiol em mulheres, o uso de anticoncepcionais, reposição hormonal, menopausa e duração do ciclo menstrual - (duração de 26-35 dias) ou fase lútea do ciclo menstrual (22-25 dias baseados em auto-relato de acordo com o primeiro dia da menstruação) – foram considerados nas mulheres. Para as mulheres na pré-menopausa, a coleta do sangue foi realizada apenas nos primeiros sete dias a partir do primeiro dia do fluxo menstrual, uma vez que neste período o nível de estrogénio não está sob a influência da fisiologia da menstruação.

Os ensaios foram realizados simultaneamente no grupo teste e controle, com as amostras de soro. Todo o pessoal do laboratório foi cego para o status dos indivíduos. Um sistema automatizado de quimioluminescência ACS:180® (Bayer Diagnostics Corp , Tarrytown , Nova Iorque) foi utilizado para medir a concentração do estradiol total em amostras de soro.

Os valores normais de estradiol foram determinados de acordo com as instruções do fabricante: menopausa (<40 pg mL^{-1}), não- gravidez (27-433 pg mL^{-1}) , e os homens (<47 pg mL^{-1}). Levando-se em conta a influência hormonal e a diferença natural entre homens e mulheres sobre os níveis séricos de estradiol, os indivíduos foram classificados como nível normal, abaixo ou acima do nível normal.

2.5 Eletromiografia de superfície

A atividade dos músculos foi analisada através de eletromiografia de superfície (EMG), por meio da avaliação bilateral dos músculos masseter, temporal anterior, esternocleidomastoideo, trapézio e deltoide, em uma amostra de 12 participantes dos grupos controle e com ambas as lesões. Os sinais EMG foram obtidos por meio de um módulo de quatro canais (EMG System do Brasil Ltda®, São José dos Campos, SP, Brasil)[38]. Foram utilizados eletrodos bipolares respeitando uma distância de 10 mm entre os mesmo e Ag 99,9% (3M do Brasil®, Sumaré, São Paulo, Brasil), juntamente com um eletrodo monopolar descartável, usado como referência (Ag/AgCl - 3M do Brasil®, Sumaré, São Paulo, Brasil) e colocado no antebraço.

Os pacientes foram orientados a permanecerem sentados em uma cadeira, com os pés afastados, os ombros relaxados e as mãos apoiadas sobre as pernas, e com a cabeça paralela ao plano de Frankfurt. Os locais de fixação dos eletrodos foram limpos com um algodão embebido em álcool a 70% para reduzir a impedância entre a pele e os eletrodos de acordo com as recomendações de SENIAM.[26] Os sinais EMG foram representados em Hz, e utilizado filtro passa-banda com frequência de corte de 20 a 500 Hz. Os sinais foram registrados por um período de 10 segundos cada. E ainda, em todos os testes, um período de cinco segundos foi selecionado (os dois segundos iniciais e três finais do sinal EMG foram descartados). O processamento do sinal foi realizado utilizando o software Windaq (Windaq/SH) (Windaq instruments®, Akron, Ohio, USA) baseando-se no protocolo de Lauriti et al.[38], por meio do cálculo do valor médio e desvio padrão de root mean square (RMS) .

Músculos masseter e temporal anterior: as avaliações dos músculos masseter e temporal anterior foram realizadas em repouso e na posição de máxima intercuspidação habitual (MIH). Para evitar o contato oclusal direto, uma fita de Parafilm M® (American National Can TM, Chicago, EUA) foi dobrada cinco vezes e disposta bilateralmente na região molar, baseado no protocolo de Lauriti et al.[38]. Os eletrodos eram fixados na região de maior protuberância dos músculos.

Músculo esternocleidomastoideo: o sinal EMG do músculo esternocleidomastóideo foi obtido pela fixação de um eletrodo posicionado ao longo de uma linha traçada a partir da fúrcula esternal ao processo mastóide, em 1/3 da

distância do processo mastóide. Para avaliação do sinal EMG, o indivíduo deveria girar o pescoço o máximo que conseguisse para o lado contra-lateral.

Músculo trapézio: a EMG do músculo trapézio superior foi registrada pela colocação de um eletrodo ao longo de uma linha imaginária que unia o acrômio e C7, a 1/3 da distância do processo acromial, devendo o participante elevar os ombros em sua posição máxima.

Músculo deltóide: atividade EMG no músculo deltóide foi medida sob completa abdução do braço com eletrodos posicionados na região de maior tônus muscular.[64]

2.6 Análise estatística

O processamento dos dados e a análise estatística foram realizadas utilizando-se o programa STATA 11.1 (StataCorp, College Station, TX, EUA). O tamanho da amostra incluiu todos os indivíduos avaliados em consultas de rotina no Centro Especializado de Ombro e Cotovelo do Instituto Nacional de Traumatologia e Ortopedia, respeitando os critérios de inclusão. As variáveis nominais foram expressas em frequências e percentuais. Para avaliar o significado das variáveis nominais entre os grupos, o teste do qui-quadrado foi utilizado. As variáveis contínuas foram expressas como média e desvio padrão. Para verificação da distribuição normal das variáveis foi utilizado o teste de Shapiro-Wilk, seguido da análise de variância com o teste t-Student e Mann-Whitney, respectivamente. Diferenças na frequência dos genótipos e alelos entre os grupos foram analisadas através do teste do qui-quadrado após a montagem para o equilíbrio de Hardy-Weinberg. Valores de $p<0.05$ foram considerados estatisticamente significativos, e os riscos associados a alelos individuais e genótipos foram calculados como a razão de chances (OR) com intervalo de confiança de 95% (IC). As variáveis que apresentaram significância estatística na análise univariada foram selecionados para introduzir um modelo de regressão logística multivariada, permitindo a exploração das diversas variáveis simultaneamente. Para calcular desequilíbrio de ligação e haplótipos, o programa de computador ARLEQUIN foi utilizado (v.20; http://anthro.unige.ch/arlequin). Correção de Bonferroni foi utilizado para corrigir as comparações múltiplas (http://www.quantitativeskills.com/sisa/calculations/bonfer.htm).

3 - ARTIGO PRODUZIDO

ESRRB POLYMORPHISMS ARE ASSOCIATED WITH COMORBID TEMPOROMANDIBULAR DISORDER AND ROTATOR CUFF DISEASE

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ABSTRACT

Temporomadibular disorder's (TMD) physical symptoms are associated with comorbid disorders. Shoulder pain is one of the main complaints of TMD patients. The purpose of this study was to investigate the association between TMD and rotator cuff disease comorbid symptoms and its biomechanical basis In this cross-sectional study, we assessed for common etiology factors of TMD and RCD in order to elucidate the TMD/RCD interaction. Orofacial (RDC-Axis I) and shoulder examinations were performed in 108 subjects. The control group consisted of 30 subjects without pain. Seventy-eight committed subjects were divided into the following groups: RCD subjects (TMD free, n=16), TMD (RCD free, n=13) and TMD/RCD affected (with RCD + TMD, n=49). A total of 8 SNPs within the *ESRRB* gene involved in the estrogen pathway were investigated. Estradiol levels were measured by chemiluminescent immunoassay. The head and cervical muscle activity was recorded with surface electromyography (EMG). Significance of nominal and continuous variables was assessed by the chi-square test and Student t-/ Mann-Whitney tests. Values of $p<0.05$ were considered significant. Multivariate logistic regression permitted the exploration of covariates simultaneously. Subjects with TMD were seven times more susceptible to RCD (OR 7.0; 95% CI, 2.7-18.4) than subjects with healthy TMJ. The risk associated with having both diseases was 6 times higher in Whites (OR, 5.9; 95% CI, 1.9-18.5) than in the control. The rs1676303 TT genotype (p=0.02) and rs6574293 GG genotype (p=0.04) are associated with RCD. TMD/RCD affected subjects showed higher association with rs4903399 (p=0.02) and rs10132091 (p=0.02). Haplotype CTTCTTAG (p=0.01) and CCTCTCAG (p=0.01) were associated with TMD/RCD. Multivariate logistic regression affirmed being White (p=0.001) as a risk factor for TMD/RCD. Estradiol levels were similar among groups. Masseter and Temporalis muscles showed lower activity in patients with both diseases in rest position (p=0.03/ p=0.02, respectively) and in the maximum clenching effort (p=0.01/ p=0.03, respectively). Sternocleidomastoid and trapezius showed lower activity in TMD/RCD affected subjects (p=0.03). Our work supported TMD as a risk factor for RCD. White ethnicity, *ESRRB* haplotypes, and low muscle surface EMG activity were identified as common biomechanical characteristics in subjects with both diseases.

INTRODUCTION

Temporomandibular disorders (TMDs) are the most common non-odontogenic pains of musculoskeletal origin[5] and affect temporomandibular joint (TMJ) and masticatory muscles.[6] TMDs are heterogeneous in presentation and multifactorial in etiology.[67] However, it has been hypothesized that the persistent pain condition observed in TMD results mainly from “central sensitization syndrome” [62] disregarding other important etiology factors, such as trauma, proinflammatory state, impaired pain regulatory mechanisms, and genetic basis.[67]

The overlap of TMD physical symptoms and other comorbid disorders, involving pains in the muscles and joints, was recently identified.[8,34,41,54] The facial pain may radiate to the surrounding ears triggering jaw pain, earache, tinnitus, headache, cervical/shoulder pain, neuralgia, and toothache.[8] Among these symptoms, shoulder pain is one of the main complaints in TMD patients.[34,54] On the other hand, the most common cause of chronic shoulder pain in adults is rotator cuff disease (RCD),[59] which is a spectrum of disorders varying from reversible tendinopathy to frank tear,[55] affecting 30% to 50% of the population.[8]

The specific etiology of a RCD has not been fully elucidated, but it has been considered to result from articular degeneration,[1] hypovascularity,[41] collagen abnormalities,[18] tensile overload,[11] and genetic factors[52] - all common to TMD development.[53,65,67]

The mechanical etiology of facial and shoulder chronic pain has been related to poor posture of the head-neck-shoulder complex.[8,53,61] However, the masticatory and cervical muscle activities, in patients with TMD associated with RCD, were not previously studied in order to elucidate the mechanic basis of these pain comorbid conditions.

Epidemiological data showed women predominantly affected by RCD[52] and TMD.[43] Possible reasons for gender differences could be explained on the basis of sex hormones and their receptors.[33] In humans, 17b-estradiol decreased sensitivity to noxious subcutaneous stimuli over the TMJ region.[67] Low estrogen or rapid changes in estrogen concentration result in an increase in articular pain,[35] explaining greater pain intensity observed in women with TMD[22,66] and RCD.[22]

Endogenous estrogen can act directly on monocytes and macrophages, increasing the production of proinflammatory cytokines which promote cartilage

resorption, inhibit synthesis of proteoglicans, and cause inflammation.[12,13] This hormone can also increase type III collagen content and lead to a decrease in the type I/III collagen ratio,[43] affecting the healing process.[44,60]

Traditionally, it has been thought that estrogen acts only through estrogen receptors α and β.[78] However, another subfamily, the estrogen-related receptors (ERRs), in the nuclear receptor subfamily, shares sequence similarity, target genes, coregulatory proteins, and action sites with estrogen receptors. This subfamily contains three members: ERR α, β and γ.[78] However, ESRRB is involved in estrogen-regulated pathways because it can bind estrogen response element, activate transcription in dependent of exogenous ligands, and share coactivators with estrogen receptors α and β.[69,76]

Many researches have associated genetic polymorphisms with musculoskeletal diseases.[10,46] There is evidence that genetic factors act as intrinsic risk factors for RCD.[23] In a recent report,[52] different mutations, functional SNP, and haplotypes in the *ESRRB* gene was associated with RCD development.[52] However, genetic effects on TMJ derangement have not been fully clarified.[2,30,48,53] Since estrogen alterations are associated with TMD, an investigation of the *ESRRB* gene may help to gain insights into the pathogenesis of TMD and explore its correlation with RCD.

Taking into account that TMD and RCD are common multifactorial diseases modulated by numerous biological processes, we hypothesized that TMD/RCD comorbidity has their etiology influenced by mechanical muscle activity, estrogen levels, and the *ESRRB* gene. Therefore, the purpose of this study was to investigate the association between TMD and RCD comorbid symptoms and its biomechanical basis. Once the diagnosis of a combined TMD and RCD is made, treatment options must be considered. Greater knowledge about these comorbid diseases may offer to help identify therapeutic targets and procedures, providing better strategies to optimize outcomes of RCD and TMD therapies.

MATERIAL AND METHODS

Subject Selection

This cross-sectional study was conducted according to recommendations from the National Institute of Traumatology and Orthopedic Research Ethics Committee

(Registration number CAAE: 07723312.0.0000.5273) and informed consent was obtained from each subject. One hundred eight subjects, from both sexes, were randomly selected from an outpatient pool during one year. Subjects answered a personal and medical history anamnesis. They underwent routine consultations in the Specialized Care Center of Shoulder and Elbow in order to evaluate shoulder and TMJ condition. Inclusion criteria for subjects were: being Brazilian, being older than 45 years, and having no previous surgery or neoplasma in TMJ/ shoulder. Patients with a history of trauma, bursitis, rheumatoid arthritis, autoimmune diseases, pregnancy, chronic use of systemic corticosteroids, and hyperlaxity were excluded. The control group consisted of 30 Brazilian subjects without pain, no signs or symptoms of TMD and RCD. Subjects diagnosed as having rotator cuff disease (RCD) and/or temporomandibular disorder (TMD) were divided into three groups: RCD subjects (TMD free, n=16), TMD subjects (RCD free, n=13), and TMD/RCD affected (with RCD + TMD, n=49). The baseline clinical parameters for the subject population are shown in Table 1.

Diagnosis of Temporomandibular Disorders

All participants were clinically examined by the same dentist (author L.L.B.) according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) Axis I[17] in order to assess the three groups of TMD: I – muscle disorder, II – disc displacement, and III –arthralgia, arthritis, arthrosis. The RDC/TMD was used to assess these three groups of TMD, using well-validated techniques, including palpation at 20 specified muscle sites.[17] Self-reported of symptoms pertaining to jaw impairment and associated pain was also gathered during the evaluation.

Clinical characteristics were noted, which included the following clinical symptoms: ear pain, toothache, burning sensation in the mouth, limited mouth opening and noises (clicking, creptation) in the TMJ, as well as diagnosed bruxism.[5] From the total, 62 subjects showed TMD.

Diagnosis of Rotator Cuff Disease

Sixty-five subjects were diagnosed with RCD. The diagnosis of RCD was based on the Motta et al.[52] protocol by clinical examination and imaging (radiography and

magnetic resonance imaging) of the involved shoulder. Subjects considered to be without RCD showed absence of history of shoulder pain, negative specific test result for impingement syndrome[56] in a complete physical examination of the shoulders, and absence of tendinopathy. All clinical evaluations were performed by one of the authors (M. V. A.) from the Specialized Care Center.

All the records of the orthopedist who carried out the RCD diagnosis and the dentist performing the orofacial examination were independent of each other.

Estrogen-related receptor β genotyping

Genomic DNA was obtained from saliva samples for all participants, as previously described.[36] The amount and purity of the DNA were determined by the use of a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All DNA samples presented $A_{260\ nm}/A_{280\ nm}$ ratios greater than 1.9.

Eight single-nucleotide polymorphisms (SNPs) in the *ESRRB* gene were selected, taking into account linkage disequilibrium relationships and structure of the genes. These SNPs had previously been identified and reported in the database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/) with minor allele frequencies>0.12. All procedures followed the STREGA reporting recommendations.[40] Details of the studied genetic variants are shown in Table 2.

Endpoint analysis of polymerase chain reactions with TaqMan chemistry (Applied Biosystems, Foster City, CA, USA) held in total 1.5 mL/reaction were used for genotyping of the selected markers in a PTC-225 tetrad thermocycler (Peltier Thermal Cycler, Bio-Rad Life Science, Hemel Hempstead, Hertfordshire, UK). Polymorphisms in the *ESRRB* gene were also analyzed in combination as haplotypes.

Stradiol Level

Endogenous estrogens are a group of steroidal compounds including 17b-estradiol, estrone, and estriol. Because the bioactivity of 17b-estradiol is the greatest of the three in vivo[16] we chose to evaluate estradiol concentration in serum.

One blood sample with 5 mL was taken from 41 subjects from the total sample group (8 controls, 9 RCD, 4 TMD, and 20 TMD/RCD affected) after 8 hours of

overnight fasting. Blood was centrifuged, and then serum samples were stored at -20^{0}C. In order to exclude the influence of hormone variables on oestradiol levels in women, the use of contraceptives, hormonal reposition, menopause, and menstrual cycle – normal length menstrual cycles (26-35 days' duration) or luteal phase of the menstrual cycle (22-25 day based purely on self-report of first day of menses) - were reported. For pre-menopausal women, blood was taken only within the first seven days from the first day of menstrual flow since in this period the estrogen level is not under the influence of the physiology of menstruation.

Assays were performed concurrently on serum specimens from cases and controls. All laboratory personnel were blinded to subjects' status. A competitive chemiluminescent immunoassay on the ACS-180 automated immunoassay system (Bayer Diagnostics Corp., Tarrytown, New York) was used to measure total oestradiol in serum samples.

The normal values of oestradiol were determined according to manufacturer's instructions: menopause (<40 pg mL^{-1}), non-pregnancy (27-433 pg mL^{-1}), and men (<47 pg mL^{-1}). Taking into account the hormonal influence and the natural difference between men and women on serum oestradiol levels, subjects were classified as normal level and below or over the normal level.

Surface Electromyography Activity

The activity of the muscles was recorded with surface electromyography (EMG) on the right and left side on masseter, anterior temporalis, sternocleidomastoid, trapezius, and deltoid muscles in a representative sample including 12 subjects from control and TMD/RCD affected groups. EMG signals were obtained by using an four-channel module (EMG System do Brasil Ltda®, São José dos Campos, SP, Brasil).[38] Surface active bipolar electrodes with 10 mm of inter-electrode distance and Ag 99.9% (3M of Brazil®, Sumaré, São Paulo, Brazil) were used, along with a disposable monopolar electrode, used as reference (Ag/AgCl – 3M of Brazil®, Sumaré, São Paulo, Brazil) and placed on forearm.

Patients were instructed to remain seated in a chair, feet apart, shoulders relaxed and hands resting on their thighs, with their heads on the Frankfurt parallel to the ground. The attachment sites of the electrodes were cleaned with a cotton ball soaked in 70% alcohol to diminish the impedance between the skin and electrodes

according to SENIAM recommendations.[26] EMG signals were sampled at 1000 Hz, band-pass from 20 to 500 Hz. The signals were recorded for 10 seconds each. In all tests, a five-second period was selected (the two initial and three final seconds of the EMG signal were discarded). The signal processing was performed using specific routines carried out in the Windaq software (WinDaq/HS) (Windaq instruments®, Akron, Ohio, USA) and were based on Lauriti et al.[38] protocol by calculating the average value and standard deviation from root mean square (RMS).

Masseter and anterior temporalis: Evaluations on masseter and anterior temporalis muscles were carried out at rest and in the maximum clenching effort (MCE). To avoid direct occlusal contact, a strip of Parafilm M® (American National Can TM, Chicago, USA) was folded five times and arranged bilaterally in the molar region based on Lauriti et al.[38] protocol.

Sternocleidomastoide: EMG from the sternocleidomastoid muscle was recorded by placing an electrode along a line drawn from the sternal notch to the mastoid process, at 1/3 the distance from the mastoid process. Subjects were asked to turn their head and neck to maximum rotation.

Trapezius: EMG from the upper trapezius muscle was recorded by placing an electrode along a line joining the acromion and C7, at 1/3 the distance from the acromion process. These steps were based on Chowdhury et al. 2013[9] protocol. Subjects must raise shoulders in their maximum position in order to analyze trapezius activity.

Deltoid: EMG activity in deltoid muscle was measured under complete arm abduction with electrodes positioned on the greatest tonus.[64]

Statistical Analyses

Data entry and statistical analysis were implemented with STATA 11.1 (StataCorp, College Station, TX, USA). The sample size included all subjects in the routine consultation in the Center of Shoulder and Elbow in the National Institute of Traumatology and Orthopaedics, respecting inclusion criteria. Nominal variables were expressed as frequencies and percentages. To assess the significance of nominal variables between groups, the chi-square test was performed. Continuous variables were expressed as mean and standard deviation. Then, after the Shapiro-Wilk test showed normal and abnormal distribution among variables, analyses of

variance with the Student t-test and Mann-Whitney test were performed, respectively. Differences in the frequency of genotypes and alleles between case and control groups were analyzed by chi-square test after fitting for Hardy-Weinberg equilibrium. Values of $p< 0.05$ were considered statistically significant, and the risks associated to individual alleles and genotypes were calculated as the odds ratio (OR) with 95% confidence intervals (CI). Variables that showed statistical significance in the univariate analysis were selected to enter a multivariate logistic regression model to permit the exploration of many covariates simultaneously and the collective effect. To calculate linkage disequilibrium and haplotypes, the computer program package ARLEQUIN was used (v.20; http://anthro.unige. ch/arlequin). Bonferroni correction was used to correct multiple comparisons (http://www.quantitativeskills.com/sisa/calculations/ bonfer.htm).

RESULTS

Clinical findings: TMD x RCD incidence

From the total of 410 subjects evaluated during one year at the Specialized Care Center of Shoulder and Elbow, 108 were included in this study. There were 80 (74%) women and 28 (26%) men, with mean age 57.2 ±8.3 years. The control group consisted of 30 subjects, 22 (73%) women and 8 (27%) men, with mean age 55±7.8 years. No difference was found among test groups (RDC, TMD, and TMD/RCD affected) when compared to the control for age, sex, smoking habits, alcohol consumption, systemic disease, diabetes, hypertension, hypothyroidism, use of calcium supplementation, analgesic, NSAIDs, SAIDs, bisphosphonate, and muscle relaxant drug.

Chi-square test revealed a higher prevalence of whites in TMD (p=0.03), RCD (p=0.003), and TMD/RCD affected (p<0.001) groups. On the basis of odds ratio calculation, the risk associated with having both diseases in Whites (OR, 5.9; 95% CI, 1.9-18.5) was six times higher than in control subjects. Additional details regarding the demographics of subjects are shown in Table 1.

During clinical TMJ and shoulder evaluations on the subjects included, the incidence of TMD was 57.4% and RCD 60.2%. From the total of patients with TMD (n=62), 75.4% (n=49) were diagnosed with RDC. The chi-square Fisher exact test

showed marked difference between RCD incidence in patients with TMD ($p<0.001$). Subjects with TMD were seven times more susceptible to RCD (OR 7.0; 95% CI, 2.7-18.4) than subjects with healthy TMJ.

According to RDC/TMD Axis I diagnosis, no difference was detected among groups with TMD. From the total of 46 subjects diagnosed as TMD, 24 subjects showed muscle disorder (Group I), 3 had disc displacement (Group II), 5 were classified with bone deficiency (Group III), and more than one diagnosis was identified in 30 subjects (Table 3).

Genetic association study

The results of the 8 SNPs in the *ESRRB* gene are provided in Table 4. As indicated by the chi-square test, statistically significant associations were observed. Allele and genotype frequencies for SNPs were within Hardy-Weinberg equilibrium in all groups. All evaluations considered the control group as the reference for statistical calculation.

Genetic difference for TMD or RCD

Significant difference in the frequency of the rs1676303 TT polymorphic genotype ($p=0.02$) between controls and RDC subjects was found. TMD subjects showed higher frequency of GG genotype ($p=0.04$) in rs6574293.

Genetic basis of TMD/RCD

Subjects with both diseases (TMD + RCD) showed significant lower frequency of polymorphic genotypes ($p=0.02$) and higher frequency of C allele ($p=0.02$) for rs4903399 when compared to controls. In RCD subjects, the presence of polymorphic genotypes ($p<0.001$) and C allele ($p=0.01$) markedly differentiated this SNP frequency from controls. In addition, rs10132091 polymorphisms ($p=0.02$) also exhibited an association with both diseases (TMD+RCD).

To assess factors concurrently, we performed a multivariate logistic regression of individual parameters considering the two main groups: controls and TMD/RCD affected diseased. The initial univariate analysis demonstrated that ethnic group

(p<0.001), rs4903399 genotypes (p=0.02), and rs10132091 genotypes (p=0.02) are potential predictive factors for TMD in association with RCD. EMG showed lower activity in masseter, temporal, sternocleidomastoid and trapezium muscle for TMD/RCD affected subjects. However, we conducted regression analysis comparing parameters which considered all patients included in these groups (genotypes and Whites). The results affirmed Whites (p=0.001) as a high risk factor associated with TMD/RCD affected subjects.

ESRRB Haplotype association with TMD/RCD affected subjects

Based on the *ESRRB* gene as a possible candidate for both diseases together, haplotype analysis was performed comparing the control group with TMD/RCD affected subjects. The summary of the haplotype analysis is shown in Table 5. There was a significant association of the haplotype CTTCTTAG (p=0.01) and CCTCTCAG (p=0.01) with the occurrence of TMD/RCD. After strict Bonferroni correction, these haplotypes kept a tendency of association with TMD/RCD affected subjects.

Estradiol serum levels analysis

Analysis of estradiol serum level considered individual sex and hormone variations. Forty-one subjects distributed in all groups were stratified as normal, below, or over levels. No statistical difference was found among groups. The frequency of TMD and/or RCD in subjects with normal values of estradiol was similar to those with below or over normal values (Table 6).

Muscle Activity (EMG)

From the 108 subjects enrolled in this study, 12 (11%) subjects (6 controls and 6 TMD/RCD affected) were submitted to EMG evaluation since 90% of patients underwent arthroplasty surgery to treat RCD. The affected side of TMD + RCD was recorded in all patients before EMG examination. TMD was affected bilaterally in five subjects and in the left side of one subject. RCD was diagnosed in two right and four left sides.

Masseter and Temporalis muscles: Right masseter and temporalis showed a significant difference between groups, showing lower activity in patients with both diseases during rest position (p=0.03/ p=0.02, respectively) and in the maximum clenching effort (p=0.01/ p=0.03, respectively). The left masseter muscle also showed lower activity during rest in the TMD/RCD affected group (p=0.01). Graphic 1 displays the RMS (root mean square) of the temporal and masseter muscles in all groups.

Cervical muscles: Taking into account head-neck rotation, there was no difference in right and left activities of the sternocleidomastoid muscle between groups, with the exception of the left sternocleidomastoid, which showed lower activity in TMD/RCD affected subjects (p=0.03). The deltoid muscle showed no activity difference between groups, while lower left trapezius activity under shoulder elevation was detected during trapezius (p=0.03) activation in TMD/RCD affected diseased subjects.

DISCUSSION

Evidence that shoulder pain is a common symptom in TMD subjects may indicate that there is a relationship between TMD and RCD. In order to elucidate this association, we conducted this study evaluating a total of 108 patients, initially divided into four groups, according to the absence or presence of TMD/RCD. Based on biomechanical aspects of each disease etiology, we analyzed the clinical and epidemiological features, the comorbid prevalence of both diseases, the surface EMG activity in facial/cervical muscles, and the *ESRRB* genetic basis.

This is the first study to show that TMD and RCD have a common genetic and mechanic basis. These diseases affect similar group of subjects, with radiated pain from TMJ to the shoulder, which can be a consequence of muscle head-neck-shoulder tension and postural changes. We speculated that estradiol levels can affect joint degeneration acting throughout ESRRB, which demonstrated to be associated with comorbid TMD/RCD development.

Subjects with TMD were seven times more susceptible to RCD than subjects with healthy TMJ. White ethnicity, *ESRRB* haplotypes, and low muscle surface EMG activity were identified as a common biological and mechanical characteristic in

subjects with both diseases. It's possible that TMD plays both a direct and indirect role in RCD development through the physiological mechanisms and by its ability to exacerbate the condition and hinder pain rehabilitation and management.

However, the complex etiopathogenesis and the variability of symptoms associated with TMD and RCD make difficult to adopt standardized diagnostic protocols.[45,52] Therefore, despite the several proposed classification systems for musculoskeletal pain,[17,47,68] our clinical diagnosis of TMD was based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD),[17] which is considered the unique system with detailed description and standardization of the clinical examination needed for TMD classification.[34] In addition, all RCD diagnosis was based on clinical and imaging examination of the involved shoulder. We believe that our data, based on a detailed diagnosis, were comprehensive enough to estimate associations between TMD and RCD.

The shoulder joint stability is dependent of the interactions and balance among multiple joints.[14,27] The imbalance between the cranium, mandible, and cervical spine relationship, as consequence of TMD, can affect functional postural and orthostatic stability, resulting in shoulder overload.[54] Forward head posture increases the strain on the posterior cervical muscles and masticatory muscle activity, which in turn causes muscle contraction and changes on the scapula positioning with consequent shoulder pain[75] and RCD development. However, no previous study analyzed the electrophysiological behavior of masticatories and cervical muscles under the influence of comorbid symptoms of TMD and RCD in order to elucidate the muscle activity interaction.

In the herein study, statistical analysis of muscles associating TMJ to shoulder showed low EMG activity in TMD/RCD affected subjects. Several studies have shown that in clenching tasks, greater masticatory muscle activity involves greater bite-force generated by the elevator muscles and greater TMJ loads.[19, 28,70,71,74] In agreement with other studies,[20,24,65] our findings showed low functional characteristics in masseter and temporal muscles of TMD/RCD subjects under rest and MCE, which would suggest a protective mechanism for damaged TMJ. It's speculated that the specific recruitment of the masseter muscle appears to be the result of descending central modulation, subsequent to nociceptive stimuli of the affected TMJ and/or myofascial, and/or periodontal nociceptors.[65] Thus, lower muscle activity could also be related to functional inability to activate the muscle

“motor drive” due to pain.

Indeed, the cervical muscle EMG showed minimal activity of sternocleidomastoid and deltoid muscles in TMD/RCD affected group. Muscles under tension increase the retention of fluids, reduce blood flow, and accumulate metabolic products,[32] which can justify less muscle function associated to radiated TMJ pain. On the other hand, low activity detected by surface EMG could mean that muscles are not only less active, but also experience incomplete activation.

Central sensitization has been the focus of recent work trying to understand pain conditions in comorbid TMD.[29,41,51] Chronic nociception and persistent stress can induce central sensitization, sympathetic upregulation, and endocrine abnormalities,[15] resulting in hyperalgesia.[37] Depletion of hormones and neurotransmitters within the hypothalamie-pituitary-adrenal-axis can lead to depression and fibromyalgia,[4] common symptoms of TMD.

Estrogen hormone action in the trigeminal ganglia, spinal cord, and medulla activates nuclear receptors increasing protein-transcription, stimulating afferent sensitivity to pain.[31,58] Therefore, it has been proposed that there is an increased relationship between circulating estrogen and joint pain,[29] which is in accordance to the related high prevalence of TMD[8] and RCD[52] in women.

Endogenous estrogen may affect the bone, cartilage, and related structures of TMJ[21,73] and may stimulate inflammatory response, resulting in internal derangement of the joint.[43] In our study, we analyzed the prevalence of comorbid TMD in both sexes showing no sex difference between subjects. In addition, estradiol levels were similar in all analyzed groups, independently of the gender. However, one limitation of this result is that it’s a cross-sectional study and sexual hormones fluctuate throughout the menstrual cycle and over months in women.[72] In addition, it’s important to consider that estrogen exerts its biological effects, in large part, through intracellular activation of its receptors[63] and through ESRRB binding.[57]

ESRRB is a group of orphan nuclear receptors that acts on establishment and maintenance of steroid producing tissues.[57] Its central DNA-binding domain is closely related to those of estrogen receptor.[3] ESRRB are expressed in tissues in which estradiol have important physiological functions and share common target genes with estrogen receptors such as osteopontin,[69] lactoferrin,[77] and pS2.[42] It has also been identified as an essential cofactor of hypoxia-inducible factor (HIF) in mediating the adaption to the hypoxic environment[3] and oxygen homeostasis.[39]

Recent studies have found evidence of classical hypoxia response pathways in tendon[7,49] and rotator cuffs express high levels of HIF.[39] These data suggest that hypoxia is a relevant damage factor in tendon injury, affected by ESRRB, and that appropriate vascular response may be essential for normal repair.[39]

RCD was recently associated with *ESRRB* CCTTCCAG haplotype in chromosome 14 and suggested as being related to tendon degeneration.[52] In contrast, no previous study analyzed *ESRRB* genetic alterations in TMD subjects. In addition, despite previous studies showing association between genetic polymorphism and TMD in many marked genes, there are no conclusive results[2,30,48] until this moment.

Taking into account the possible influence of *ESRRB* genetic polymorphism on joint degeneration, our study evaluated 8 SNPs in the *ESRRB* gene in subjects with and without TMD/RCD. Our findings clearly showed significant association between *ESRRB* polymorphisms and TMD and/or RCD. From the total analyzed *ESRRB* SNPs, four regions showed disease association. *ESRRB* rs1676303 polymorphism was related to RCD while rs6574293 showed relation with TMD and RCD separately. However, *ESRRB* rs4903399 polymorphisms were highly incident in subjects showing both diseases. In fact, these results show a common genetic basis associated with the *ESRRB* gene for TMD and RCD. Considering that nuclear receptors are outstanding for drug discovery, our findings offer insights into the HIF-ESRRB pathway for TMD/RCD therapy.

Investigating the influence of biomechanical factors with respect to those disorders is an effort to develop more effective treatment programs. Particularly, for these subjects, the treatment of TMD should be part of a multidisciplinary therapy, which often requires cooperation with other medical and dentistry specialties. A better knowledge of the biomechanical basis involved in TMD/RCD comorbidity may provide new therapeutic approaches and the on-time intervention for joint healing.

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Table 1. Baseline clinical parameters of the studied population.

Variables	Control N=30 30 (%)	RCD N=16 16 (%)	TMD N=13 13 (%)	TMD/RCD affected N= 49 49 (%)	Healthy TMJ With RCD	TMD Without RCD	TMD/RCD affected
					p-value (OR (CI))		
Ethnic group							
Whites	11 (36,6)	13(81,2)	11(84,6)	38(77,5)	**0.03** (7.4 (1.4-42.7)	**0.003** (9.5 (1.5-46.3)	**0.0002** (5.9 (1.9 -18.5)
Non-w hites	19 (63,3)	3(18,7)	2(15,3)	11(22,4)			
Age (years)	55 ± 7,8	57,2 ± 8,2	56,3 ± 8,1	57,3 ± 8,0	0.06	0.4	0.57
Sex							
Female	22 (73,3)	11(68,7)	9(69,2)	38(77,5)	0.74 (0.8 (0.1-3.6)	0.7 (0.8 (0.1-4.3)	0.67 (1.26 (0.3-4.0)
Male	8 (26,6)	5(31,2)	4(30,7)	11(22,4)			
Smoking							
Non-smoking	26 (86,6)	13(81,2)	13(100)	44(89,7)	0.6 (0.6 (0.1-4.5)	0.1 (---)	0.67 (1.3 (0.2-6.5)
Smoking	4 (13,3)	3(18,7)	0(0)	5(10,2)			
Alcohol consumption							
Non-drinking	21 (70)	13	11	36	0.4 (1.8 (0.3-10.6)	0.3 (2.3 (0.3-19.0)	0.7 (1.1 (0.3-3.6)
Drinking	9 (30)	3	2	13			
General medical condition							
Systemic disease	30 (100)	16(100)	13(100)	46(93,8)	0.3	1.0	0.1 (0.0 (0.0-3.7)
Diabetes	5 (16,6)	2(12,5)	5(38,4)	10(20,4)	0.7 (0.7 (0.0-5.1)	0.1 (3.1 (0.5-17.5)	0.6 (1.2 (0.3-4.9)
High blood pressure	20 (66,6)	5(31,2)	5(38,4)	32(65,3)	**0.02** (0.2 (0.0-0.9)	0.08 (0.3 (0.0-1.4)	0.9 (0.9 (0.3-2.7)
Hypothyroidism	1 (3,3)	2(12,5)	1(7,6)	1(2,0)	0.2 (4.1 (0.2-127.0)	0.5 (2.4 (0.0-98.2)	0.7 (0.6 (0.0-23.1)

CI: Confidence interval; TMD: Temporomandibular disorder; RCD: Rotator Cuff Disease

Table 2 - Genetic variants in the *ESRRB* gene.

SNP	Chromosome	Base Pair Position	Base Change*	MAF**	SNP Type	Nearest gene locus
rs4903399	Ch14	76775202	C>T	0.1	intergenic	intergenic
rs1077430	Ch14	76897677	C>T	0.3	intron	*ESRRB*
rs2860216	Ch14	77006008	C>T	0.3	intergenic	intergenic
rs10132091	Ch14	76870818	C>T	0.4	intron	*ESRRB*
rs1676303	Ch14	76992164	C>T	0.2	intergenic	intergenic
rs745011	Ch14	76917275	C>T	0.4	intron	*ESRRB*
rs4903419	Ch14	76984655	A>G	0.1	intergenic	intergenic
rs6574293	Ch14	76870600	A>G	0.1	intron	*ESRRB*

*Base change according to Applied Biosystems; **MAF: minor allele frequency according to GenBank.

Table 3. Characteristics in all subjects with TMD according to Axis I RDC/TMD evaluation.

Parameters	With TMD N=62		*p*-value*	Odds Ratio	95% CI
	Without RCD N=13	With RCD N=49			
Muscle disorders (Group I)	7	17	0.2	0.4	(0.1-1.8)
Disc displacement (Group II)	1	2	0.5	0.5	(0.0-15.5)
Arthralgia, arthritis, and arthrosis (Group III)	0	5	0.2	--	---
Multiple diagnoses	5	25	0.4	1.6	(0.4-6.9)

CI: Confidence interval; TMD: Temporomandibular disorder; RCD: Rotator Cuff Disease

Tabela 4. *ESRRB* genotype and alleles distribution in all subjects.

SNP	Genotypes	Control N=30 30 (%)	RCD N=16 16 (%)	TMD N=13 13 (%)	TMD/RCD affected N= 49 49 (%)	p-value* (CI)		
						Healthy TMJ With RCD	*TMD Without RCD*	TMD/RCD affected
rs4903399	CC-CT-TT	14-13-2	14-2-0	6-6-1	36-12-1	**0.031**	0.9	0.07
	CT+TT	15	2	7	13	**0.009** (0.1 (0.0-0.8)	0.8 (1.0 (0.24-4.91)	**0.02** (0.3 (0.1-0.9)
	C	41	30	18	84	**0.01** (6.2 (1.2-42.2)	0.2 (2.3 (0.5-11.6)	**0.02** (2.4 (1.0-5.9)
	T	17	2	3	14			
rs1077430	CC-CT-TT	6-14-9	4-9-3	2-5-6	11-21-17	0.6	0.6	0.8
	CT+TT	23	12	11	38	0.5 (0.7 (0.1-4.1)	0.6 (1.4 (0.2-12.3)	0.8 (0.9 (0.2-3.1)
	C	26	17	9	43	0.4 (1.3 (0.5-3.6)	0.3 (0.6 (0.2-1.8)	0.9 (0.9 (0.4-1.9)
	T	32	15	17	55			
rs2860216	CC-CT-TT	3-14-12	3-4-9	2-8-3	4-16-29	0.2	0.5	0.3
	CT+TT	26	13	11	45	0.4 (0.5 (0.0-3.7)	0.6 (0.6 (0.0-6.4)	0.7 (1.3 (0.2-7.6)
	C	20	10	12	24	0.7 (0.8 (0.3-2.3)	0.3 (1.6 (0.5-4.6)	0.1 (0.6 (0.2-1.3)
	T	38	22	14	74			
rs10132091	CC-CT-TT	8-16-5	4-7-5	2-5-6	4-25-20	0.5	0.1	**0.02**
	CT+TT	21	12	11	45	0.8 (1.1 (0.2-5.7)	0.3 (2.1 (0.3-17.2)	**0.02** (4.2 (1.0-19.4)
	C	32	15	9	33	0.4 (0.7 (0.2-1.8)	0.08 (0.4 (0.1-1.2)	**0.008** (0.4 (0.2-0.8)
	T	26	17	17	65			
rs1676303	CC-CT-TT	1-7-21	2-9-5	1-1-11	4-17-28	**0.02**	0.4	0.3
	CT+TT	28	14	12	45	0.2 (0.2 (0.0-4.0)	0.5 (0.4 (0.0-17.3)	0.4 (0.4 (0.0-4.1)
	C	9	13	3	25	**0.007** (3.7 (1.2-11.4)	0.6 (0.7 (0.1-3.2)	0.1 (1.8 (0.7-4.7)
	T	49	19	23	73			
rs745011	CC-CT-TT	9-13-7	7-9-0	3-2-8	12-26-11	0.09	0.05	0.7
	CT+TT	20	9	10	37	0.3 (0.5 (0.1-2.4)	0.5 (1.5 (0.2-8.9)	0.5 (1.3 (0.4-4.3)
	C	31	23	8	50	0.0 (2.2 (0.8-6.2)	0.5 (0.3 (0.1-1.1)	0.7 (0.9 (0.4-1.8)
	T	27	9	18	48			
rs4903419	AA-AG-GG	20-8-1	13-3-0	9-4-0	32-17-0	0.5	0.7	0.3
	AG+GG	9	3	4	17	0.3 (0.5 (0.0-2.6)	0.9 (0.9 (0.1-4.9)	0.7 (1.1 (0.4-3.5)
	A	48	29	22	81	0.3 (2.0 (0.4-10.1)	0.8 (1.1 (0.2-4.9)	0.9 (0.9 (0.3-2.5)
	G	10	3	4	17			
rs6574293	AA-AG-GG	0-11-18	0-5-11	0-1-12	0-10-39	0.6	**0.04**	0.09
	AG+GG	29	16	13	49	---	---	
	A	11	5	1	10	0.6 (0.7 (0.2-2.8)	0.06 (0.1 (0.0-1.4)	0.1 (0.4 (0.1-1.3)
	G	47	27	25	88			

*** p-value compared to control group (without both diseases)**

CI: Confidence interval; TMD: Temporomandibular disorder; RCD: Rotator Cuff Disease

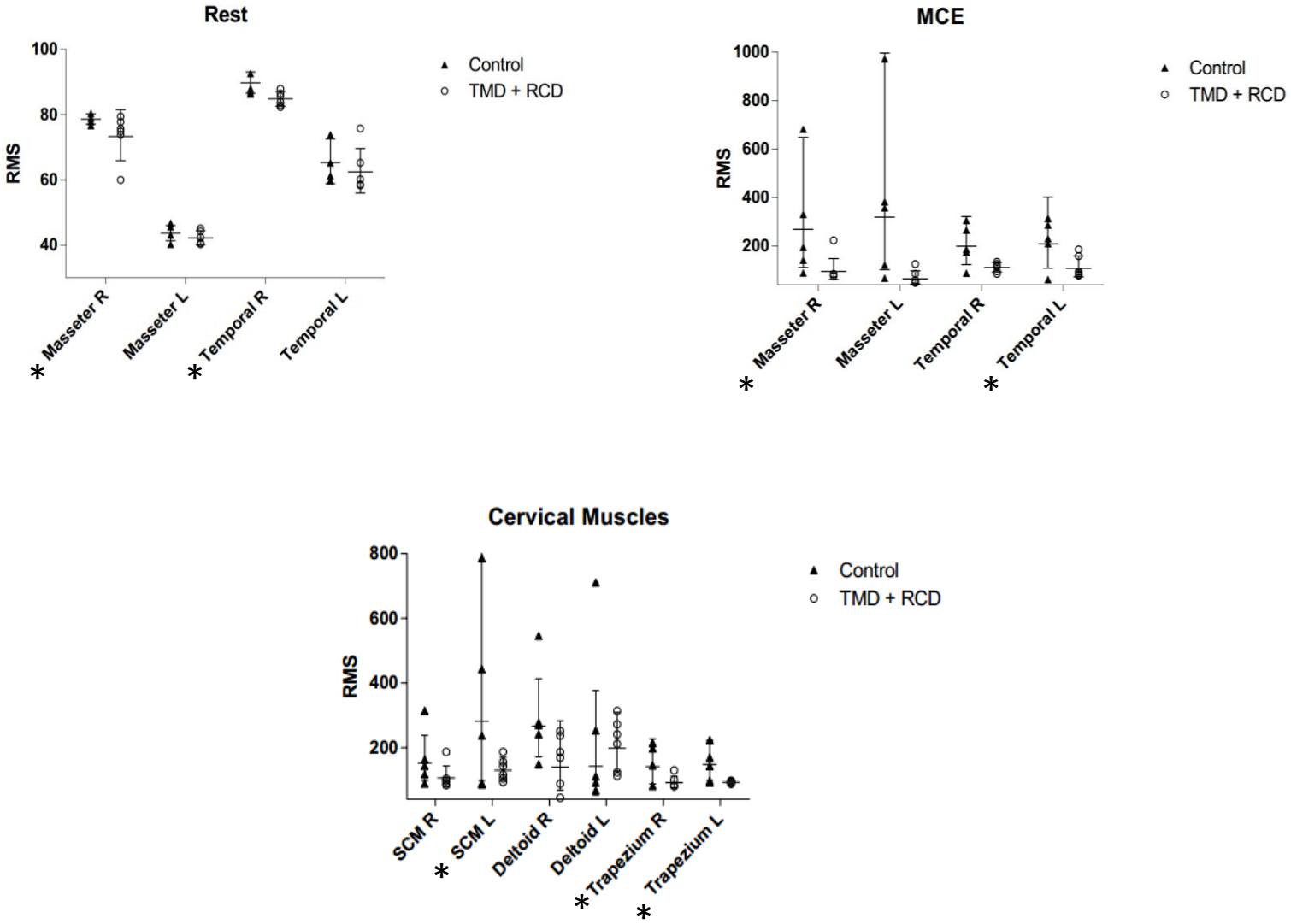
Tabela 5. Haplotypes analyses in *ESRRB* gene.

Gene	Haplotypes	Frequency		*p*-value	Bonferro Correction p-value
		Control (n=30)	TMD/RCD affected (n=49)		
ESRRB - Ch14 (rs4903399; rs1077430; rs2860216; rs10132091; rs1676303; rs745011; rs4903419; rs6574293)	CTTTTTAG	0.12	0.1	0.90	0.5
	CTTCTTAG	0.07	0.1	**0.01**	0.07
	CCTCTCAG	0.10	0.04	**0.01**	0.07
	CTTTTTGG	0	0.08	1	1.0
	TTCTTTAG	0	**0.05**	0.1	0.6

Table 6. Stradiol levels.

Stradiol level*	Control group N=8	RCD N=9	TMD N=4	TMD/RCD affected N= 20	p-value* (compared to controls)		
					Healthy TMJ With RCD	*TMD Without RCD*	*TMD With RCD*
Below	0	0	0	2			
Normal	7	9	4	16	0.2	0.4	0.6
Above	1	0	0	2			

TMD: Temporomandibular disorder; RCD: Rotator Cuff Disease



Graphic 1. Surface EMG activity from head and cervical muscles. A. Masseter and Temporal EMG in rest position: note the lower activity of right muscle in TMD/RCD affected. B. Masseter and Temporal EMG in maximum clenching effort (MCE): most of the evaluated muscle showed lower EMG activity in TMD/RCD affected subjects. C. EMG from cervical muscle: lower EMG activity was detected in sternocleidomastoid (SCM) and Trapezium in TMD/RCD affected group. TD (TMD/RCD affected); * ($p<0.05$ were considered significant).

4 - CONCLUSÕES

1. A DTM é um fator de risco para o desenvolvimento da LMR.

2. Polimorfismos no gene *ESRRB* estão relacionados ao desenvolvimento de DTM e LMR.

3. Baixa atividade eletromiográfica é uma consequência à presença de DTM e LMR em conjunto.