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THE POLYMORPHISM OF METALLOPROTEINASES 1 AND 13 AND POSTTRAUMATIC ELBOW STIFFNESS

O POLIMORFISMO DAS METALOPROTEINASES 1 E 13 E A RIGIDEZ ARTICULAR PÓS-TRAUMÁTICA DO COTOVELO

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ABSTRACT

Introduction: To evaluate the relationship between the genetic polymorphism of matrix metalloproteinases 1 and 13 and post-traumatic elbow stiffness, as well as the association of other risk factors with this condition. Materials and methods: We evaluated 20 patients with posttraumatic elbow stiffness and 12 controls with traumatic elbow disorders without contracture. Deoxyribonucleic acid (DNA) was obtained from buccal mucosa epithelial cells of the volunteers. The MMP-1 and MMP-13 genotypes were determined using PCR-restriction fragment length polymorphism assays. Results: We did not find any significant differences in the frequency of genotypes and alleles between the test and control groups for the polymorphism of metalloproteinases 1 and 13. We observed that genotypes 1G/2G and 2G/2G of MMP-1 were present in 65% (13/20) of patients with articular stiffness and 50% (6/12) of controls (p = 0.599). Genotypes A/A and A/G of MMP-13 were obtained in 95% (19/20) of patients and 91.6% (11/12) of controls (p = 0.491). Among the prognostic factors for elbow stiffness, only immobilization time correlated positively. The mean immobilization time for cases and controls were 16 ± 10 days and 7 ± 7 days, respectively (p = 0.017). Conclusion: The genetic polymorphism of MMP-1 at position -1607 and MMP-13 at position -77 was not associated with post-traumatic elbow stiffness. ***Level of Evidence III; Prognosis Study; Case-Control Study.***

Keywords: Articular rigidity. Elbow. Capsule contracture. Trauma. Metalloproteinases. Genetic polymorphism.

RESUMO

Introdução: Avaliar a relação entre o polimorfismo genético das metaloproteinases 1 e 13 da matriz e a rigidez pós-traumática do cotovelo, assim como a associação de outros fatores de risco com essa condição. Material e método: Foram avaliados 20 pacientes com rigidez pós-traumática do cotovelo e 12 controles com distúrbios traumáticos do cotovelo sem contratura. O ácido desoxirribonucleico (DNA) de voluntários foi obtido a partir de células epiteliais da mucosa bucal. Os genótipos MMP-1 e MMP-13 foram determinados usando ensaios de polimorfismo de comprimento de fragmento de restrição de PCR. Resultados: Não encontramos diferença significativa na frequência de genótipos e alelos entre os grupos teste e controle para o polimorfismo das metaloproteinases 1 e 13. Observamos que os genótipos 1G/2G e 2G/2G de MMP-1 estavam presentes em 65% (13/20) dos pacientes com rigidez articular e 50% (6/12) dos controles (p = 0,599). Os genótipos A/A e A/G da MMP-13 foram obtidos em 95% (19/20) dos pacientes e 91,6% (11/12) dos controles (p = 0,491). Dentre os fatores prognósticos para rigidez de cotovelo, apenas o tempo de imobilização se correlacionou positivamente. O tempo médio de imobilização para casos e controles foi de 16 ± 10 dias e 7 ± 7 dias, respectivamente (p = 0,017). Conclusões: O polimorfismo genético de MMP-1 na posição -1607 e MMP-13 na posição -77 não foi associado à rigidez pós-traumática do cotovelo. ***Nível de Evidência III; Estudos Prognósticos; Estudo de Caso-Controle.***

Descritores: *Rigidez articular. Cotovelo. Contratura capsular. Trauma. Metaloproteinases. Polimorfismo genético.*

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INTRODUCTION

Elbow joint stiffness after trauma is a challenging complication in orthopedic practice and it is believed that soft tissue contracture, especially of the joint capsule, is the main source of stiffness. The inflammatory process initiated after the trauma may cause the contraction of elbow capsule due to an action of myofibroblasts and metalloproteinases of matrix 1 and 13 (MMP-1 and MMP-13).[1] MMPs are a family of zinc-dependent endopeptidases, capable of degrading almost all elements of the extracellular matrix. The expression of these proteases is regulated at different levels,

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including genetic transcription. Some genetic single nucleotide polymorphisms (SNPs) in the gene promoting region of MMPs are correlated with an increase in the activity and production of these enzymes.[2] Recently, MMP-1 and -13 SNPs have been associated with diseases that cause joint stiffness, such as Dupuytren's disease[3] and adhesive capsulitis.[4] However, to date, we have not found any study evaluating the influence of genetic polymorphisms on posttraumatic elbow stiffness.

Our primary objective was to evaluate the relationship of genetic polymorphism in the promoter region of the genes of matrix metalloproteinases 1 and 13 with posttraumatic joint stiffness of the elbow. The secondary objective of our study was to assess other risk factors that could be associated with the development of this disease.

MATERIALS AND METHODS

Study design, participants and eligibility

We performed a case-control study. Patients with traumatic elbow disorders were selected during treatment follow-up between July 2017 and December 2018 in a single center. The study was approved by the local ethics committee.

The study patients had all the following inclusion criteria: skeletal maturity; interval greater than 6 months between the initial trauma and the evaluation for eligibility in the study; previous elbow fracture (including the distal humerus, proximal ulna, radial head) or reduced elbow dislocation, treated both surgically and non-surgically; physiotherapy treatment to gain range of motion for 4 months or more. Patients with umeroulnar joint incongruity, intra-articular screw penetration, heterotopic ossification, moderate or severe elbow osteoarthritis (Type II and III according to Rettig and Hastings classification)[5] were not included. In addition, we did not include patients with previous or active infection in the elbow, fracture nonunion, with a history of traumatic brain injury, neurological injury in the affected limb, associated ipsilateral upper limb fracture, previous elbow surgery, autoimmune systemic diseases, refusal or difficulty to understand the terms of the study and use of immobilization on the elbow for longer than 1 month.

The case group consisted of patients with range of motion - flexion-extension $<100^{o}$, and control group consisted of those with a functional range of motion (greater than 30^{o} to 130^{o}). Measurement of patients' range of motion was performed with a goniometer by the research assistant.

Variables

The following patient data were collected using a standardized form by the main author: age, sex, ethnicity, dominant side, affected side; clinical comorbidities, trauma type (low or high energy, elbow immobilization time, type of treatment performed (surgical or non-surgical), time of physical therapy treatment and fracture type (simple or comminuted).

DNA extraction and obtaining the genotype

DNA from epithelial buccal cells was extracted using the procedure described by Aidar and Line[6] DNA concentration was estimated by optical density measurements 260/280 nm. The researchers group who did the genetic analysis of the collected material, was blinded to which group the patient was included.

The MMP genotypes were determined using the PCR-random fragment length polymorphism assays (Table 1). PCR primers were done in a total volume of 15 μL containing 100 ng of genomic DNA, 8 μL Taq Green (Amersham Pharmacia-Biotech, Uppsala, Sweden), and 200 nmol of each primer. An 8 μL aliquot of PCR products then was digested with 1 unit of specific enzyme overnight.

The total amount of aliquot of the digest was electrophoresed on 5% agarose at 20 mA. The gel was stained by GelRed™ (Biotium Inc, Fremont, CA, USA).

The SNPs were identified previously and included in the database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/) with minor allele frequencies greater than 0.2. The MMP-1 polymorphism at position -1607 (rs1799750) is characterized by the insertion a base guanine (G), which results in two alleles: 1G and 2G. The allele 2G is represented by the DNA of 118 base pairs and the allele 1G is represented by the DNA of 89 base pairs. The heterozygous genotype has a combination of both alleles. The 2G allele is associated with increased transcriptional activity of this gene and degradation of the extracellular matrix.[7]

The MMP-13 polymorphism at position -77 (rs2252070) is characterized by an exchange of adenine (A) for guanine (G) creating two different alleles (A or G). Allele A is represented by a band of DNA with 445 base pairs and the allele G by two bands of DNA with 248 and 197 base pairs. The heterozygous genotype has a combination of both alleles. This polymorphism was associated with an alteration in transcription activity: the A allele has approximately twice the transcription activity, than the G allele.[8]

Sample size

The sample size calculation used a level of significance of 5% and power of 80%. At the time this research protocol was elaborated, there were no studies, to our knowledge, regarding the association between the genetic polymorphism of MMPs and posttraumatic elbow stiffness. Using a rate of 25% of the allele 2G of MMP-1 in the control group, an expected difference of 53% between groups based on study by Godoy et al.,[7] 32 individuals were required in the study.[8]

Statistical Analysis

We submitted continuous variables to the assessment of normality, using the Kolmogorov-Smirnov test, and homogeneity, using the Levene test. Continuous variables are presented by means and standard deviation, while categorical variables are in absolute and percentage values. The comparison between cases and controls, with respect to the different variables, was performed using Chi-square or Fisher's exact tests, in the categorical variables. In continuous variables, using the Wilcoxon test. The difference in the frequency of alleles and genotypes of MMP-1 and 13 in individuals with post-traumatic elbow stiffness and controls was evaluated using the Chi-square test.

The software ARLEQUIN Version 2.0 (Laurent Excoffier CMPG, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland)[9] was used for the analysis of Hardy-Weinberg equilibrium

Table 1. PCR-RFLP conditions for MMP-1 (rs1799750) and MMP-13 (rs2252070) polymorphisms.

SNP	Primers (5'-3')	Anealling	RFLP	pb PCR-RFLP
MMP-1	F: TCGTGAGAATGTCTTCCCATT	55°C	XmnI	118 (Allele 2G)
(rs1799750)	R: TCTTGGATTGATTTGAGATAAGTGAAATC	30 s	37°C	89 + 29 (Allele 1G)
MMP-13	F: GATACGTTCTTACAGAAGGC	53°C	BsrI	445 (Allele A)
(rs2252070)	R: ACAAATCATCTTCATCACC	1 min	65°C	248 + 197 (Allele G)

PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; MMP = matrix metalloprotease; SNP = single nucleotide polymorphism; F = primer forward; R = primer reverse; bp = base pairs.

in the population studied. The distribution of genotypes of the study subjects was in the Hardy-Weinberg equilibrium. For the data analysis, we used SPSS® Version 21.0 (IBM Corp, Armonk, NY, USA) with a level of significance of 5%.

RESULTS

We evaluated 32 patients, 20 with posttraumatic elbow stiffness and 12 without stiffness (Table 2). There was no loss of samples during DNA extraction and genotyping, and the distribution of the study participants' genotypes was in Hardy-Weinberg equilibrium. The cases and controls groups had a mean age of 39.3 ± 11 and 47.4 ± 12.6, respectively ($p = 0.068$). Of the patients who had elbow stiffness, 14 (70%) were males, and of the control group, 6 (50%) were males ($p = 0.258$). The groups did not differ regarding ethnicity, dominant side affected and clinical comorbidities ($p > 0.999$, $p = 0.647$ and $p = 0.695$, respectively). The mean of immobilization time was 15.7 ± 10.7 days in the case group and 6.9 ± 7 days in the control group ($p = 0.017$). We observed that the 1G allele of the MMP-1 genetic polymorphism (rs1799750) was more frequent in the case and control groups with a frequency of 79% and 65%, respectively (p = 0.477). Regarding the genotypes, we observed that the genotypes 1G / 1G and 1G / 2G were present in 95% of the patients in the elbow stiffness group and 92% of the patients without stiffness (p = 0.599). Also, we found no significant difference in the frequency of the genotypes and alleles of the MMP-13 genetic polymorphism (rs2252070) between the case and control groups (Tables 3 and 4).

DISCUSSION

Joint stiffness is a frequent complication of elbow trauma, and about 10 to 15% of patients will need some type of surgery during the treatment, once a 50° reduction in elbow mobility can result in up to 80% of functional loss.[10] The most common cause of elbow stiffness is the contraction of soft tissues after the initial injury, which worsens with the use of prolonged immobilization.[11] The elbow capsule becomes thick and there is an increase in the amount of type I, III and V collagen fibers, proteoglycans and MMP-1 and 13, as well as the formation of myofibroblasts in a posttraumatic elbow stiffness.[1,12]

Table 2. Baseline demographic and clinical characteristics.

	Cases	Controls	p
Age (years)	39.3 11	47.4 12.6	0.068
Sex			
Male	14 (70%)	6 (50%)	0.258
Female	6 (30%)	6 (50%)	
Ethnicity			
White	10 (50%)	6 (50%)	0.999
Nonwhite	10 (50%)	6 (50%)	
Dominant side affected			
Yes	10 (50%)	7 (58%)	0.647
No	10 (50%)	5 (42%)	
Clinical comorbidities			
Yes	5 (25%)	4 (33%)	0.695
No	15 (75%)	8 (67%)	
Trauma type			
Low energy	8 (40%)	8 (67%)	0.273
High energy	12 (60%)	4 (33%)	
Treatment type			
Surgical	11 (55%)	8 (67%)	0.713
Non-surgical	9 (45%)	4 (33%)	
Orthopedic injury type			
Simple fracture	9 (45%)	8 (66%)	0.398
Comminuted fracture	6 (30%)	3 (25%)	
Dislocation	5 (25%)	1 (8%)	
Elbow immobilization time(days)	15.7 10.7	6.9 7	0.015
Time of physical therapy treatment (weeks)	38.8 32.6	16 21	0.040

Table 3. Distribution of the MMP-1 and MMP-13 allele in case and control groups.

	Cases	Controls	p
MMP-1			
1G	26 (65%)	19 (79%)	0.497
2G	14 (35%)	7 (21%)	
MMP-13			
A	30 (75%)	15 (63%)	0.289
G	10 (25%)	9 (37%)	

Table 4. Distribution of the MMP-1 and MMP-13 genotype in case and control groups.

	Cases	Controls	p
MMP-1			
1G/1G	7 (35%)	6 (50%)	0.599
1G/2G	12 (60%)	5 (42%)	
2G/2G	1 (5%)	1 (8%)	
MMP-13			
A/A	11 (55%)	4 (33%)	0.491
A/G	8 (40%)	7 (58%)	
G/G	1 (5%)	1 (8%)	

In our study, the genetic polymorphism of metalloproteinases 1 (rs1799750) and 13 (rs2252070) was not associated with post-traumatic elbow stiffness. It was not possible to find a statistically significant difference in the frequency of alleles and genotypes in the group of cases and controls of the two metalloproteinases studied. Genetic polymorphism is a variation in the sequence of DNA nucleotides and is present in over 1% of the population. Among these variations, the most common is the SNPs.[13] Some SNPs in the promoter region of MMP genes are correlated with an increase in the activity and production of these enzymes[2,14] As we did not find a direct association between the genetic polymorphism of MMP-1 and 13 with posttraumatic elbow stiffness, we believe that the increase in levels of metalloproteinases in these patients is related to other biological causes resulting from the trauma, and not to the genetic influence. The role of several external factors that affect both transcriptional and post-transcriptional activation of MMPs has been demonstrated, which include inflammatory cytokines, hormones and growth factors (TGF-β, EGFR, TNF-α, IL-1β).[15]

The genetic polymorphisms of MPPs are associated with several orthopedic diseases, such as posterior tibial tendinopathy, rotator cuff tear, stiffness after rotator cuff repair, adhesive capsulitis and Dupuytren's disease.[3,4,7,16,17] The balance of the Extracellular matrix (ECM) of the articular capsule, tendons and ligaments is guaranteed by equal rates of deposition and removal of collagen fibers, the latter activity mediated by MMPs. An imbalance can lead, when withdrawal predominates, to tendinopathies and tears, such as that of the posterior tibial tendon[7] and rotator cuff.[16] When deposition predominates, we can observe fibrotic processes such as Dupuytren's disease[3] and adhesive capsulitis of the shoulder.[4]

In contrast, we found association between prolonged immobilization and posttraumatic stiffness. Patients with posttraumatic elbow stiffness remained immobilized for 16 days on average during treatment,

whereas patients without stiffness were immobilized for only 7 days ($p = 0.017$). Monument et al.[18] have shown that prolonged joint immobilization results in numerous physical and biochemical disorders in and around joint structures, including erosions of the articular cartilage, decreased articular proteoglycan content, changes in collagen fibers and synovial adhesions. Others authors such as Schollmeier et al.,[19] showed that immobilization favored contracture of the capsule and reduced the volumetric capacity of the joint. The ideal immobilization time for healing, and the correct time to start the range of motion gain are critical points in the treatment of elbow injuries.

Our study has some limitations. The number of study participants is small for a genetic study. However, elbow stiffness is an uncommon condition and we noticed that patients who evolved without stiffness stopped having follow-up. The power of the study was 21.2% for the evaluation of MMP-1 polymorphism and 18.8% for MMP-13. With these low values, we cannot completely refute the hypothesis that these polymorphisms may influence the development of elbow stiffness. Functional genomics studies carried out to date in orthopedic diseases have been small or modest in scale and they are restricted in terms of power, although necessary to demonstrate experimental feasibility and provide first insights into disease biology.[20] In addition, we selected a very heterogeneous group of patients with regard to age, type of fracture and types of treatment. The disadvantage in this is the lack of pairing between patients in the groups, which increases the possibility of confusion bias. However, the external validity of the study has increased.

We could highlight that this study is the first approaching the association of the genetic polymorphism of matrix metalloproteinases with posttraumatic elbow stiffness. Genetic polymorphism of MMPs were associated with increased risk of post-operative stiffness susceptibility and severity in patients after rotator cuff repair.[17] We understand that a larger scale investigation is necessary, trying to decipher if there is really a genetic influence in this disease. The identification of patients most susceptible to elbow stiffness, may in the future lead to alternative forms of treatment and increase knowledge of pathophysiology and the factors involved in the development of posttraumatic elbow stiffness.

CONCLUSION

The genetic polymorphisms of MMP-1 at position -1607 (rs1799750) and MMP-13 at position -77 (rs2252070) were not associated with posttraumatic elbow stiffness. However, among the risk factors evaluated, prolonged immobilization was correlated with stiffness of the joint elbow after trauma.

AUTHORS' CONTRIBUTION: GMRP: conceived and planned the activities that led to the study, wrote the Original Draft and participated in the review process of the manuscript; JHA: conceived and planned the activities that led to the study, development the design of methodology, interpreted the results of the study, participated in the review process of the manuscript; MCLGS: provided study material and scientific support; ALGS: development the design of methodology, provided study material and scientific support; MECG: participated in the review process of the manuscript; EAM: participated in the review process of the manuscript; FBAS: participated in the review process of the manuscript; AAFN: supervision of the study, participated in the review process of the manuscript. All authors have read and approved the final article, and also authorized its publication.

REFERENCES

1. Hildebrand KA, Zhang M, Hart DA. High rate of joint capsule matrix turnover in chronic human elbow contractures. Clin Orthop Relat Res. 2005.439:228-34.
2. Rutter JL, Mitchell TI, Butticè G, Meyers J, Gusella JF, Ozelius LJ, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res. 1998.58:5321-5.
3. Ulrich D, Ulrich F, Piatkowski A, Pallua N. Expression of matrix metalloproteinases and their inhibitors in cords and nodules of patients with Dupuytren's disease. Arch Orthop Trauma Surg. 2009.129:1453-9.
4. Cohen C, Leal MF, Loyola LC, Santos SEB, Ribeiro-dos-Santos ÂKC, et al. Genetic variants involved in extracellular matrix homeostasis play a role in the susceptibility to frozen shoulder: a case-control study. J Orthop Res. 2019.37:948-56.
5. Rettig LA, Hastings H 2nd, Feinberg JR. Primary osteoarthritis of the elbow: lack of radiographic evidence for morphologic predisposition, results of operative debridement at intermediate follow-up, and basis for a new radiographic classification system. J Shoulder Elbow Surg. 2008.17:97-105.
6. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J. 2007.18:148-52.
7. Godoy-Santos A, Cunha MV, Ortiz RT, Fernandes TD, Mattar R Jr, dos Santos MC. MMP-1 promoter polymorphism is associated with primary tendinopathy of the posterior tibial tendon. J Orthop Res. 2013.31:1103-7.
8. Yoon S, Kuivaniemi H, Gatalica Z, Olson JM, Butticè G, Ye S, et al. MMP13 promoter polymorphism is associated with atherosclerosis in the abdominal aorta of young black males. Matrix Biol. 2002.21(6):487-98.
9. Schneider S, Roessli D and Excoffier L. Arlequin: a software for population genetics data analysis. User manual ver 2.000. [Internet]. Geneva: Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva; 2009. [accessed 2020 17 jun]. Available at: http://dna.ac/filogeografia/labs/Lab_SIMCOAL_Topicos_Crawford_2009.pdf.
10. Guglielmetti CLB, Gracitelli MEC, Assunção JH, Andrade-Silva FB, Nicolosi-Pessa MM, et al. Randomized trial for the treatment of post-traumatic elbow stiffness: surgical release vs. rehabilitation. J Shoulder Elbow Surg. 2020.29:1522-9.
11. Born CT, Gil JA, Goodman AD. Joint contractures resulting from prolonged immobilization: etiology, prevention, and management. J Am Acad Orthop Surg. 2017.25:110-6.
12. Hildebrand, KA, Zhang M, Van Snellenberg W, King GJW, Hart DA. Myofibroblast numbers are elevated in human elbow capsules after trauma. Clin Orthop Relat Res. 2004.(419):189-97.
13. Brookes AJ. The essence of SNPs. Gene. 1999.234:177-86.
14. Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J Cell Physiol. 2007.211:19-26.
15. Gaffney J, Solomonov I, Zehorai E, Sagi I. Multilevel regulation of matrix metalloproteinases in tissue homeostasis indicates their molecular specificity in vivo. Matrix Biol. 2015.44-46:191-9.
16. Assunção JH, Godoy-Santos AL, Dos Santos MCLG, Malavolta EA, Gracitelli MEC, Ferreira Neto AA. Matrix metalloproteases 1 and 3 promoter gene polymorphism is associated with rotator cuff tear. Clin Orthop Rel Res. 2017.475:1904-10.
17. Ling Y, Peng C, Liu C, Zhang N, Yue S. Gene polymorphism of IL-6 and MMP-3 decreases passive range of motion after rotator cuff repair. Int J Clin Exp Pathol. 2015.8:5709-14.
18. Monument MJ, Hart DA, Salo PT, Befus AD, Hildebrand KA. Posttraumatic elbow contractures: targeting neuroinflammatory fibrogenic mechanisms. J Orthop Sci. 2013.18:869-77.
19. Schollmeier G, Uhthoff HK, Sarkar K, Fukuhara K. Effects of immobilization on the capsule of the canine glenohumeral joint. A structural functional study. Clin Orthop Relat Res. 1994.(304):37-42.
20. Steinberg J, Zeggini E. Functional genomics in osteoarthritis: Past, present, and future. J Orthop Res. 2016.34(7):1105-10.