PROFILE OF SEVERAL AUTOANTIBODIES, COMPLEMENT AND VITAMIN D LEVELS IN BRAZILIAN PATIENTS WITH CHIKUNGUNYA INFECTION

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Abstract. Introduction. Chikungunya virus infection (CKV) may lead to chronic arthritis in up to 40% of the patients. There are previous data regarding positive auto antibodies in CKV patients. Objective is to systematically evaluate the prevalence of auto antibodies in CKV patients. Methods. All study participants had clinical manifestations being CKV positive at least serologically or by RT-PCR data. The following auto antibodies were assessed: antinuclear antibodies (ANA), anti-dsDNA, anti-Sm, anti-Ro/SS-A, anti-La/SS-B, anti-U1RNP, IgG and IgM anticardiolipin, anticyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF), antiribosomal P protein, lupus anticoagulant, anti-Jo-1 and anti-Scl-70. CH100, C3 and C4 complement components, serum levels of immunoglobulins, C-reactive protein, erythrocyte sedimentation rate, alpha1-acid glycoprotein, antithyroglobulin, antithyroperoxidase, TRAb, 25 hydroxyvitamin D (25OHD), dengue and zika IgG and IgM antibodies were also measured. Results. 30 CKV patients were included. Mean age was 59.1±15.7 years, 85% females and 77% Caucasian subjects. Disease duration comprised 4.9±4.0 months. Oligoarthritis was observed in 93% cases. Mean C-reactive protein levels were 10.1±6.8 ng/dL, erythrocyte sedimentation rate — 34.2±19.9 mm/1st hour and alpha1-acid glycoprotein 115.5±52.6 mg/dL. Intramuscular betamethasone depot single dose injection was administered in 54%, prednisone — in 15% and methotrexate — in 23% cases. Importantly, 1/30 (3.3%) cases was positive for ANA, 4/30 (13.3%) — for RF and none was positive for anti-CCP or any other auto antibodies. Complement and immunoglobulin levels were all within the normal range. Low levels of 25OHD were observed in 88% cases.

Key words: Chikungunya, autoantibodies, autoimmunity, arboviruses, rheumatoid arthritis.
ПРОФИЛЬ УРОВНЕЙ НЕКОТОРЫХ АУТОАНТИТЕЛ, КОМПЛЕМЕНТА И ВИТАМИНА D У БОЛЬНЫХ С ИНФЕКЦИЕЙ ЧИКУНГУНЬЯ В БРАЗИЛИИ
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Резюме. Введение. Инфекция вирусом чикунгуния (CKV) может приводить к хроническому артриту у 40% пациентов. Существуют ранее полученные данные об обнаружении аутоантител при CKV-инфекции. Цель — оценка наличия аутоантител у пациентов с CKV.

Методы. У всех участников исследования были отмечены клинические проявления CKV-инфекции, получены положительные результаты, по крайней мере, серологического анализа или ОТ-ПЦР на CKV и выявлены следующие аутоантитела: антинуклеарные (ANA), анти-dsDNA, анти-Sm, анти-Ro/SS-A, анти-La/SS-B, анти-U1RNP, антикардиолипин IgG и IgM, антициклический цитруллинированный пептид (anti-CCP), ревматоидный фактор (RF), антитела к антирибосомному белку P, волчаночный антикоагулянт, анти-Jo-1 и анти-Scl-70. Также исследовались уровень компонентов комплемента C3 и C4, общая активность комплемента (CH100), сывороточные уровни иммуноглобулинов, С-реактивный белок, скорость оседания эритроцитов, альфа-кислотный гликопротеин, антитиреоглобулин, антитиреопероксидаза, TRAb, 25-гидроксивитамин D (25OHD), антитела к вирусам денге и Зика.

Результаты. В исследование было включено 30 пациентов с CKV. Средний возраст составлял 59,1±15,7 лет, 85% составляли женщины, 77% — представители европеоидной расы. Давность заболевания составила 4,9±4,0 мес. Олигоартрит отмечен у 93% обследованных. Средние уровни С-реактивного белка составляли 10,1±6,8 нг/дл, СОЭ — 34,2±19,9 мм/ч и альфа-1-кислотный гликопротеин — 115,5±52,6 мг/дл. Внутримышечная инъекция депо бетаметазона в разовой дозе применялась в 54% случаев, преднизон — в 15% и метотрексат — в 23%. Важно отметить, что 1 проба из 30 (3,3%) была положительной на ANA, 4 из 30 (13,3%) — на RF, и ни одна не была положительной на анти-CCP или любые другие аутоантитела. Уровни комплемента и иммуноглобулинов были в пределах нормы. Низкие уровни 25OHD наблюдались в 88% случаев. Заключение. Хотя мы наблюдали высокий процент суставных проявлений у пациентов с CKV, настоящее исследование обнаружило низкую частоту аутоантител у пациентов с CKV.

Ключевые слова: Чикунгуния, аутоантитела, аутоиммунитет, арбовироз, ревматоидный артрит.

Introduction

The Chikungunya virus (CKV), a member of the Togaviridae family, Alphavirus genus, was first isolated from humans in 1952, in the coastal area of Muawiya, Makondo and Rondo, currently known as Tanzania. Brazil had a large outbreak of CKV between September 2014 and 2017. Until the 37th epidemiological week, 236,287 probable cases of CKV were evaluated and 116,523 were serologically confirmed [4]. In the literature, from 14.4% to 87.2% of these cases may evolve to chronic joint involvement [9].

Previous data have found some percentage of autoantibodies in CKV. In this research line, antinuclear antibodies (ANA), rheumatoid factor, and anti-cyclic citrullinated peptide (anti-CCP) were previously studied in CKV scenario [2].

Our group has recently described the characteristics of 63 CKV patients from Brazilian Amazon, which the main arthritis pattern identified was oligoarticular, with a predominance of ankles and knees, and also with a chronicity rate of 48%, however no autoantibody was studied. Until now, we do not have any knowledge of studies in which several autoantibodies profile in CKV were systematically analysed [11].

Therefore, this study aims to systematically investigate the profile of several autoantibodies in patients with confirmed CKV infection.

Methods

All study participants had clinical manifestations (fever, rash and arthritis) and positivity for at least serology (IgG and/or IgM) or RT-PCR fulfilling the diagnosis of CKV infection [14]. Anti-Chikungunya IgG and IgM antibodies were detected using a single-serum monoclonal antibody-capture enzyme-linked immunosorbent assay (ELISA) technique. This research is characterized as a descriptive cross-sectional study that was conducted at a private clinic in Salvador, Bahia State, Brazil. All patients had negative IgM serology for dengue since this arbovirus is an important differential diagnosis.

All patients agreed to participate and were requested to sign an informed consent of participation.

Laboratory evaluation. All tests were collected before treatment. Antinuclear antibodies were detected by indirect immunofluorescence (IIF) using HEP-2 cells as substrate. Anti-dsDNA antibodies were also detected by IIF using Crithidia lucilae. The presence of IgG and IgM anticytobiolin antibodies (aCL) was
analyzed by padronized ELISA, and positive values were considered above 10GPL and 10MPL, respectively. The following antibodies anti-Ro/SS-A, anti-La/SS-B, anti-U1RNP and anti-Sm were performed using ELISA and immunodiffusion; anti-CRP by chemiluminescence with microparticles; RF by nephelometry and normal values were considered below 141U/mL; antiribosomal P by ELISA; lupus anticoagulant by functional assays (activated partial thromboplastin time and diluted Russell viper venom time); anti-Jo-1 and anti-Scl-70 by ELISA and double immunodiffusion; and antithyroglobulin, antithyroperoxidase and TRAb by competitive immunoassay and electrochemiluminescence. Total hemolytic complement activity (CH100) was measured by immunohemolysis assay (normal range 150—350 units/ml). Serum levels of C3 and C4 complement components were determined by immunodiffusion test (Dade Behring Marburg, Marburg, Germany). C3 normal range is 84—167 mg%, and C4 normal range is 16—31 mg%. The erythrocyte sedimentation rate was measured by Westergren method and C-reactive protein by nephelometry. 25OHD was measured by chemiluminescence. Low levels of this vitamin were considered when lower than 20 ng/mL. Serum levels of IgG, IgA and IgM immunoglobulins were measured by nephelometry and IgE by immunofluorimetry. 

Chikungunya detection Anti-Chikungunya Virus ELISA (IgG and IgM) (Euroimmun, Germany) was used to detect antibodies to CKV. 

Viral RNA was extracted using a QIAmp Viral Mini Kit (Qiagen, Inc., USA), following the manufacturer’s instructions and amplified by reverse transcription polymerase chain reaction (PCR) using primers for CHIKV according to previously described protocols. In summary, the RT-PCR assay for detection of CHIK was realized using the GoTaq™Probe1-Step RT-qPCR System (Promega) following recommendations of the manufacturer. The reaction was carried out using an ABI 7500 Real Time PCR system. The processing of all samples included negative and positive controls as well as internal controls (Ribonuclease P, RNase P) to ensure reliability of the reaction. 

Statistical analysis. Results were expressed as mean± standard deviations (SD), median (range) or frequency as percentage. Windows Excel was the used program. 

Results 

The main data is summarized in the Table. Mean age of all 30 CKV patients was 59.1±15.7 years, 85% were female and 77% were caucasian while 33% were black or mulatto. The disease duration was 4.9±4.0 months. Comorbidities were reported in 50% of patients and were: osteoarthritis in 50%, systemic hypertension in 25%, obesity in 25% and diabetes in 13%. Nine out 26 (35%) had previously used non-steroidal anti-inflammatory drugs. Oligoarthritis was observed in most of the cases (93%) and polyarthritis in 7%. Ankles followed by the knees were the most common involved joints.

Serology (IgG and or IgM) for CKV was positive in 25 (83.32%) and one patient had positive RT-PCR for CKV at inclusion. Median levels of antibodies to CKV were IgG CKV 15.0 (0—198) and IgM CKV 5.42 (0.293—34.8). All patients were negative for IgM dengue antibodies, but 19% had positive IgG. IgG Zika positivity was detected in 1/30 patient. Ultrasensitive C-reactive protein levels were 10.1±6.8 ng/dL and erythrocyte sedimentation rate 34.2±29.9 mm/1st hour. 

Intramuscular betamethasone depot injection in a single dose was administered in 54%, oral prednisone initiated in 15% and methotrexate in 23% of the cases. Importantly, 1/30 (3.3%) was positive for ANA, 4/30 (13.3%) for RF and none was positive for anti-CCP or any other autoantibodies (anti-dsDNA, anti-Ro/SS-A, anti-La/SS-B, anti-RNP, anti-Sm, antiribosomal P, anti-CCP, lupus anticoagulant, IgG and IgM anticardiolipin, anti-Jo-1, anti-Scl-70 and anti-thyroid antibodies). Titles of RF were very low and were: 17.61, 16.88, 16.30 and 15.7IU/mL. Complement and immunoglobulin levels were all within the normal range. Low levels of 25OHD was observed in 88% of the cases.

Discussion 

To our knowledge, this is the first study to systematically evaluate the prevalence of several autoantibodies in patients with CKV infection using highly specific methods. The pathogenesis of arthropathy following

| Table. Demographic, clinical and treatment features of the 30 patients with Chikungunya arthritis |
|-----------------------------------------------|
| **Features** | **CKV patients (n = 30)** |
| Age, years | 59.1±15.7 |
| Caucasian race | 77% |
| Female gender | 88% |
| Disease duration, months | 4.9±4.0 |
| Diabetes mellitus | 13% |
| Systemic arterial hypertension | 25% |
| Obesity | 25% |
| Osteoarthritis | 50% |
| Oligoarthritis | 93% |
| Polyarthritis | 7% |
| C-Reactive protein, mg/dL | 10.1±6.8 |
| ESR*, mm/1st hour | 34.2±29.9 |
| IgG CKV* median levels (range) | 15.0 (0—198) |
| IgM CKV* median levels (range) | 5.42 (0.293—34.8) |
| Oral prednisone | 15% |
| Intramuscular betamethasone use | 54% |
| Methotrexate use | 23% |
| 25OHD* low levels | 88% |
| ANA* | 3.3% |
| Rheumatoid factor | 13.3% |

Note. Values are expressed in mean± SD or percentage (%).

ANA: antinuclear antibodies; CKV: Chikungunya infection; ESR: erythrocyte sedimentation rate; 25OHD: 25-hydroxyvitamin D.
infection with CKV is not completely clear; however, based on studies conducted with other arthritogenic viruses, arthritis possibly occurs due to the virus' ability to replicate and mediate the production of tissue damage in joints, which in turn depends on the host immune response [12].

In previous studies, some patients met the international classification criteria for rheumatoid arthritis and spondyloarthritis in the chronic phase of the disease [7]. In addition, there are reports of exacerbation of cutaneous psoriasis and of onset of psoriasis after CKV [6]. In a study that compared untreated rheumatoid arthritis and CKV patients using cyto metric analysis of blood mononuclear cells, the authors found higher percentages of activated and effector CD4+ and CD8+ T lymphocytes in CKV and also in rheumatoid arthritis than in healthy controls. In conclusion, CKV and RA patients have similarities regarding clinical features and lymphocyte activation [10].

Furthermore, another article which described 21 new cases of RA following CKV infection and observed autoantibodies. Interesting that after 21 months, 81% had erosions and importantly rheumatoid factor were positive in 57% and anti-CCP in 28% of these cases [2]. Autoimmunity in CKV was reviewed by Tanay in 2017 [13].

A large cross-sectional study was conducted in Colombia with 907 patients with clinically confirmed (n = 424) or laboratory-confirmed (n = 483) chikungunya virus infection. Of these patients, 65 were randomly selected for eligibility screening, of whom 38 were eligible for study participation in the chronic arthritis group. Synovial fluid analysis revealed no detectable chikungunya virus. Thus, we can assume that the virus can cause arthritis by an autoantibody induction mechanism in infected patients [3].

Recently, Cavalcanti et al. have described increased serum levels of galactin-9 in patients with Chikungunya fever [5]. Galectins are a family of animal lectins with an affinity for beta-galactosides, that have multiple functions including working as receptors in innate immunity and as a control for inflammatory responses in both innate and adaptive immunity. Galectins regulate functions of immune cells, such as lymphocytes and macrophages, which have a main role in the chikungunya inflammatory process. These authors suggested galactin-9 may play a role in the immunopathogenesis of the inflammatory process in chikungunya fever, as morning stiffness may reflect the systemic inflammatory process.

Regarding the antibodies profile in CKV patients, previous studies have evaluated a few numbers of autoantibodies, almost exclusively RF, ANA and anti-CCP. In this line, most of these studies does not describe the laboratory methodology used to detect the autoantibodies. In just a few the methodology was stated using more modern techniques such ELISA for anti-CCP and nephelometry for RF [2, 8]. No autoantibodies were found in the Blettery study, including 147 patients [4]. In Bouquillard et al. study, with 21 patients, the authors found a high positivity for RF of 57.1%, anti-CCP 28.6% and 9.5% for ANA [2].

Antinuclear antibodies were evaluated in 2 studies involving CKV patients being found a positivity from 0 to 30%, on the other hand, the frequency of rheumatoid factor and anti-CCP ranged from 0 to 57.1% and 0 to 56.5% in 8 studies, respectively. Therefore, the real frequency of positivity for autoantibodies was currently unknown. Giving the past studies on the theme, our study differential includes systematic evaluation of several autoantibodies' profile in CKV patients with arthropathy referred to rheumatology service, using highly specific methods for antibodies detection.

We have found a prevalence of 11.5% of rheumatoid factor in our CKV population what is higher than the described in the literature (about 1–2%) on healthy people. In clinical practice, this fact can make the differentiation between CKV arthropathy and rheumatoid arthritis triggered by virus infection even harder. On the other hand, the presence of anti-CCP should strengthen the suspicion of RA, as well as the presence of bone erosions, as shown in other studies [9]. The main strength of this study relies on its originality about the wide variety of autoantibodies studied in these patients. The use of standardized and recommended techniques in the guidelines for the measurement of autoantibodies is another original point. Moreover in the presence of varied rheumatic manifestations that may happen in the chronic phase of CKV, the positivity of the specific autoantibodies evaluated in this study could help to investigate differential diagnosis, since low positivity found in our population, is an auxiliary element in decision making when added to clinical evaluation. The limitations of this study also need to be addressed. First, the number of patients included was relatively small; second, we did not standardize a specific time after the onset of CKV infection to include patients. Maybe if we performed the autoantibody evaluation in a long-term CKV infection, the positivity for autoantibodies could increase.

Conclusion

This study suggests that the presence of autoantibodies in patients with Chikungunya in about 4 months of infection onset is low. Mainly represented by ANA and RF positivity when standard techniques with good specificity are performed. However, future studies including many participants are needed to confirm the present data.

Highlights

- Chikungunya virus infection may induce autoimmunity;
- We observed a low frequency of autoantibodies in this study with 13.3% of rheumatoid factor and 3.3% of antinuclear antibodies.
- No patient had positivity for anti-CCP or evolved with a fullblown autoimmune rheumatic disease.
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