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HELICOBACTER PYLORI AND t(11;18)(q21;q21) TRANSLOCATION IN GASTRIC MALT LYMPHOMA

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ABSTRACT – ***Context*** - Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is clearly associated with *Helicobacter pylori* gastritis and can be cured with anti- *H pylori* therapy alone. The presence of t(11;18)(q21;q21) translocation is thought to predict a lower response rate to anti- *H pylori* treatment. ***Objective*** - To study the presence of t(11;18)(q21;q21) genetic translocation and its clinical impact in low-grade gastric MALT lymphoma Brazilian patients. ***Methods*** - A consecutive series of eight patients with gastric MALT lymphoma were submitted to gastroscopy, endoscopic ultrasound, histopathological examination, *H pylori* search and RT-PCR-based methodology. All patients received anti-*H pylori* treatment. Eradicated patients were followed-up every 3-6 months for 2 years. ***Results*** - Eight patients were studied. All patients had tumor involvement restricted to the mucosa or submucosa and seven patients had low-grade gastric MALT lymphoma. All infected patients achieved *H pylori* eradication. Histological tumor regression was observed in 5/7 (71%) of the low-grade gastric MALT lymphoma patients. The presence of t(11;18)(q21;q21) translocation was found in 4 (57%) of these patients; among them only two had histological tumor regression following *H pylori* eradication. ***Conclusion*** - RT-PCR is a feasible and efficient method to detect t(11;18)(q21;q21) translocation, being carried out in routine molecular biology laboratories. The early detection of such translocation can be very helpful for better targeting the therapy to be applied to gastric MALT lymphoma patients.

HEADINGS - Lymphoma B-cell marginal zone. *Helicobacter pylori*. Genetic translocation. Reverse transcription. Polymerase chain reaction.

INTRODUCTION

Gastric mucosa-associated lymphoid tissue (MALT) lymphomas (GML) represent 2% to 8% of gastric neoplasms, in general. They comprise the second most frequent gastric neoplasm[3, 13, 25]. The normal stomach is virtually unprovided with lymphocytes and plasmatic cells[13]. Few lymphocyte collections may be found in the gastric body, but never featuring germinal centers. The suggestion that chronic gastritis secondary to infection by *Helicobacter pylori* (HP) could be associated with primary gastric lymphoma derived from the studies involving multiple gastric biopsies that feature the presence of lymphoid tissue in a large part of individuals infected by HP[11, 35]. In 1991, Wotherspoon et al.[32], in England, in a histological review of 110 GML cases found the presence of HP infection in 92% of the cases. Subsequently, consistent epidemiologic evidences also confirmed the association of GML with previous HP infection[24].

Once in vitro studies demonstrated that the proliferation of low-grade GML was stimulated, via T lymphocytes and cytokines, by specific HP strains[12], different studies confirmed, in vivo, that once the bacteria is eradicated, the stimulation mediated by T-cells of the tumor is ceased, resulting in remission of the GML[10, 23, 28, 33, 34]. A recent systematic review involving 32 studies on 1408 patients with low-grade GML, restricted to the mucosa and submucosa, showed the presence of HP infection in 88.2% of the cases and complete remission of the tumor simply by bacteria eradication in more than 75% of the cases[37]. A recently held consensus meeting, specifically focused on GML, recommends the HP eradication as the first line of treatment for GML[26].

However, approximately 25% of patients with early lesions restricted to the mucosa and submucosa showed no tumor remission following the eradication of the bacteria and, in this case, it is speculated that there is a non-identified high grade component

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in the lymphoma or, yet, the presence of cytogenetic alterations. Specific molecular events have been associated with the development of GML. Among them, the expression of Bcl10 protein and chromosomal translocations[4], especially t(11;18)(q21;q21), which represents the most common rearrangement associated with GML[2, 31]. Studies have suggested that the presence of such translocations could lead to an independence of the lymphoma cells to the antigenic stimulus, thus constituting a predictive factor of tumor response to anti-HP therapy. Therefore, its presence should be indicative of a possible lack of tumor regression simply with HP eradication, requiring the adoption of other therapeutic alternatives such as surgery, radio or chemotherapy[1, 16, 17, 27]. The tumor remission in response to the anti-HP therapy composes a slow histological process that could be as long as 1 to 2 years[10]. The detection of the presence of chromosomal translocation as an indicator of a bad response to the antibacterial treatment could reduce the treatment period so that patient could be faster referred to other forms of treatment.

Translocation t(11;18)(q21;q21) occurs between chromosomes 11 and 18 producing the fusion of the API2 gene, located in chromosome 11q21, with the MLT (MALT lymphoma - associated translocation) gene, known as MALT1, located in chromosome 18q21[15]. The most frequent breakpoint for the AP12 gene is in the API2-Downstream from exon 7 (E7), located before the CARD domain in 93% of the cases. The MALT1 gene breakpoints are located in the carboxyl terminal, MALT1-Upstream from four exons (E3, E5, E8 and E9) (Figure 1), being the interaction between the E7 of the API2 gene and the E5 of the MALT1 gene the most frequent fusion[36]. Whenever the gene fusion occurs, the API2 gene free amine group (N-terminal) binds to the MALT1 carboxyl group (C-terminal) and yields a chimeric "fusion product" called API2-MALT1[18].

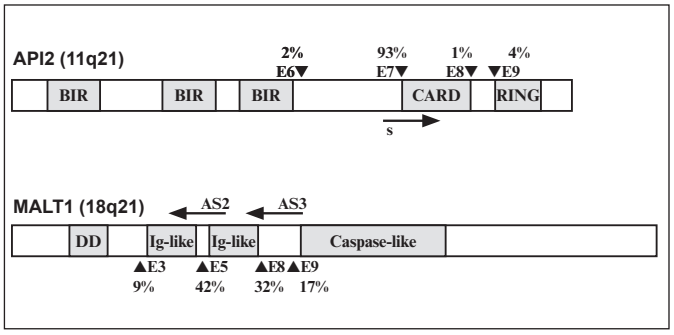

FIGURE 1. Representation of the API2 and MALT1 genes involved in t(11;18). Vertical arrows represent the known breakpoints and their incidence in percentages. Horizontal arrows indicate the position of sense (S) and anti-sense (AS) primers for PCR (adapted from ref. 8).

The aim of this study is to evaluate the presence of the t(11;18)(q21;q21) chromosomal translocation in low-grade GML Brazilian patients and its clinical impact on the the evolution of these patients.

METHODS

Patients

From 2009 to 2010, a consecutive series of eight patients with GML were included in the study. They had been referred to the Alfa Institute of Gastroenterology at the Clinical Hospital of UFMG in Belo Horizonte to undergo endoscopic ultrasound for tumor staging. The study was approved by UFMG's Research Ethics Committee (ETIC-UFMG 216/09) and all patients recruited signed an informed consent.

Methods

All patients included in the study were submitted to the following examinations: gastroscopy and endoscopic ultrasound to analyze the tumor involvement of gastric layers using Ann Arbor Staging System, as modified by Musshoff, for MALT lymphomas in the gastrointestinal tract[20]; histopathological examination according Isaacsson & Norton classification[14]; HP infection search using, at least, one diagnostic method: urease test and/or histological survey using Giemsa staining and/or a carbon-13 labeled urea breath test (UBT) as previously validated[5], and, molecular studies using specific primers for API2-MALT1 (see below).

All patients received anti-HP treatment combining omeprazole 20 mg, clarithromycin 500 mg and amoxicillin 1.0 g taken twice daily for 7 days. A new upper digestive endoscopy and/or UBT were performed 2 months after treatment to confirm eradication. Patients still infected were retreated with the association of omeprazole 20 mg, levofloxacin 250 mg and amoxicillin 1.0 g taken twice daily for 10 days, and a new endoscopy and UBT were carried out to confirm eradication. Following eradication, endoscopic examinations were carried out every 3-6 months during 2 years to monitor the tumor evolution, adopting histological criteria suggested, in France, by the Adult Lymphoma Study Group (GELA)[6].

Molecular studies

1. RNA extraction

To study the API2-MALT1 translocation the samples of tumor region were collected with forceps model Radial Jaw® from Boston Scientific (USA) compatible with endoscope with working channel of 2.8 mm, were immediately preserved in RNAlater® (Ambion Cat. # AM7020) and forwarded to the laboratory for conditioning at 4°C overnight and then stored at -80°C. The recovery of samples stored in RNAlater® was conducted at room temperature and the tissue disruption in liquid nitrogenor manually. The extraction of total RNA was performed with Trizol® reagent (Invitrogen Cat. # 15596-026) and RNA extracted quantified by Nanodrop (Thermo Scientific). To eliminate any genomic DNA traces, the samples were purified with DNase enzyme using the RQ1 RNase-Free DNase kit (Promega Cat. # M6101). After treatment with DNase samples were submitted to phenol:chloroform reaction for removal of salts, and stored at -80°C.

2. Study of API2-MALT1 translocation by RT-PCR

Reverse transcription followed by polymerase chain reaction (RT-PCR) was conducted based on the RETROscript® kit (Ambion Cat. # AM1710) with sample pre-heating to 85°C to denature secondary structures which could inhibit the action of the enzyme RT, since it is a sequence rich on C≡G bindings. Short sequences of specific reverse primers of the API2-MALT1 gene (AS2 (50μM) and AS3 (50μM)) and constitutive gene S15 (AS (50μM)) as positive control (+) were used for cDNA synthesis for greater specificity of the methodology[17, 18] (Table 1). The reverse transcription reaction was catalyzed by the enzyme M-MuLV (Moloney Murine Laeukemia Virus - MMLV-RT). The samples were incubated at 50°C for 1 hour for annealing of primers and cDNA synthesis, followed by incubation at 92°C for 10 min to inactivate the RT enzyme.

Amplification was separately performed for each pair primers forward and reverse (Table 1) using the enzyme SuperTaq™/DNA Polymerase (Ambion Cat. # AM2052). For S15 gene PCR, which evaluated the presence of cDNA in the samples, the reaction was standardized based on RETROscript® kit. Amplification of the gene API2-MALT1 was based on the studies of Liu et al.[17, 18], in which primer was added to the samples at a concentration of 100μM. The thermal cycling for API2-MALT1 were divided into steps of denaturation at 94°C to separate the double-stranded; annealing of the primers to the cDNA template strand with the use of a touchdown PCR with an initial temperature of annealing at 65°C and progressive reduction of 1°C per cycle until reaching 58°C, which was maintained for an additional 35 cycles; and amplification (extension) at 72°C for DNA polymerase activity. The touchdown PCR optimizes the process and provides more specificity to the reaction.

Amplified samples were stored at -20°C for later use or immediately used in routine methodology of electrophoresis on a 2% agarose gel. The gel bands were observed in UV transilluminator and recorded using GelDoc-It TS 310 Imaging System – UVP.

RESULTS

Eight patients, six of them women and two men averaging 57 years of age (39-73) constituted the study sample. All the patients had dyspeptic symptoms with a variable endoscopic appearance, especially ulcers and/or infiltrative lesions. The endoscopic ultrasound showed tumor involvement restricted to the mucosa or submucosa (stage I or IE of the Ann Arbor Staging System) in all eight patients. Histopathological examination confirmed the presence of low-grade GML in seven patients and high-grade GML in one. HP infection was confirmed in all but one patient (patient F008). The bacteria was eradicated by administration of a single anti-HP regimen in five patients, while a second antibacterial regimen was needed to achieve eradication for other two patients. Table 2 shows demographic data on the eight patients and their clinical outcome.

TABLE 1. Main characteristics of forward (sense [S]) and reverse (anti-sense [AS]) primers used for the study

Gene - Primer	Sequence of Primer	PCR products (bs)
API2-MALT1*		
S	5'-GGA AGA GGA GAG AGA AAG AGC A- 3'	
AS2	5'-GGA TTC AGA GAC GCC ATC AA- 3'	67; 340
AS3	5'-CAA AGG CTG GTC AGT TGT TT- 3'	73; 100; (133; 197; 230); 409
S15		
S	5' -TTC CGC AAG TTC ACC TAC C- 3'	361
AS	5' -CGG GCC GGC CAT GCT TTA CG- 3'	

* The primers of API2-MALT1 gene were designed according to Liu et al. [18] and verified on database of genetic sequences GenBank® in API2 (NM_001165) and MALT1 (AB026118) genes, available by The National Center for Biotechnology Information (NCBI).

TABLE 2. Demographical data and clinical evolution of the eight GML patients

Patient	Sex/Age (years)	GML Histology	Clinical Evolution
F001	F/73	Low grade	Underwent two anti-HP treatments for eradication of the microorganism. Histological regression of tumor.
F002	M/40	Low grade	HP was eradicated with single therapeutic regime. Histological regression of tumor.
F003	M/73	Low grade	HP was eradicated with single therapeutic regime. Histological regression of tumor.
F004	F/41	High grade	HP was eradicated with single therapeutic regime. No histological regression of tumor occurred and patient was referred to oncological treatment.
F005	F/66	Low grade	Underwent two anti-HP treatments for eradication of the microorganism. Histological regression of tumor.
F006	F/38	Low grade	HP was eradicated with single therapeutic regime. No histological regression of tumor occurred and patient was referred to oncological treatment.
F007	F/51	Low grade	HP was eradicated with single therapeutic regime. Histological regression of tumor.
F008	F/73	Low grade	Anti-HP treatment was done though its presence was not attested. Evolved to a more advanced stage and patient was referred to oncological treatment.

F: Female; M: Male; GML: gastric MALT lymphoma; HP: *H. pylori*.

Synthesis of cDNA with specific primers led to the S15 gene detection in all samples (Figure 2). The use of an API2 (S) gene initiator which covers 93% of the known API2 breakpoints and two MALT1 gene initiators (AS2 and AS3) which covers the main MALT1 breakpoints (Figure 1) detected API2-MALT1 translocation in four of the eight patients studied (50%) (Figures 3 and 4). The full-length transcript found characteristic for AS2 has between 300 and 350 base pairs (bp) (Figure 3) and between 400 and 450 bp for AS3 (Figure 4). The absence of bandsin the gel for API2-MALT1 refers to patients with no translocation.

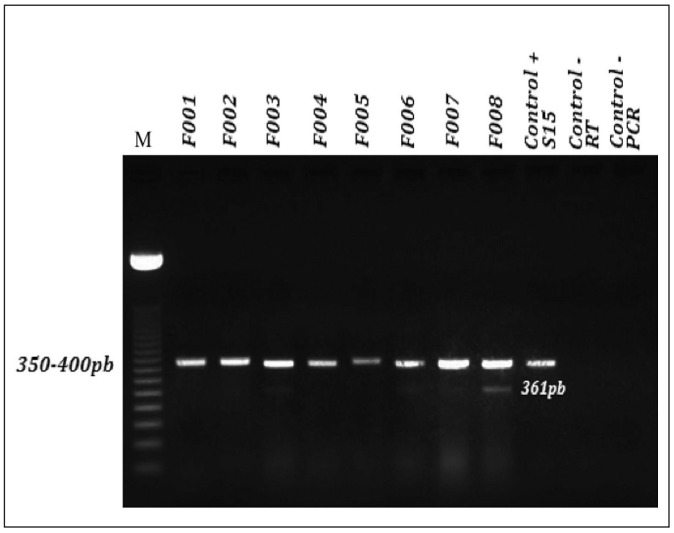

FIGURE 2. Agarose gel electrophoresis of the amplified products by RT-PCR obtained with S15 gene oligonucleotides in the study samples. M, molecular-weight marker (50 bp).

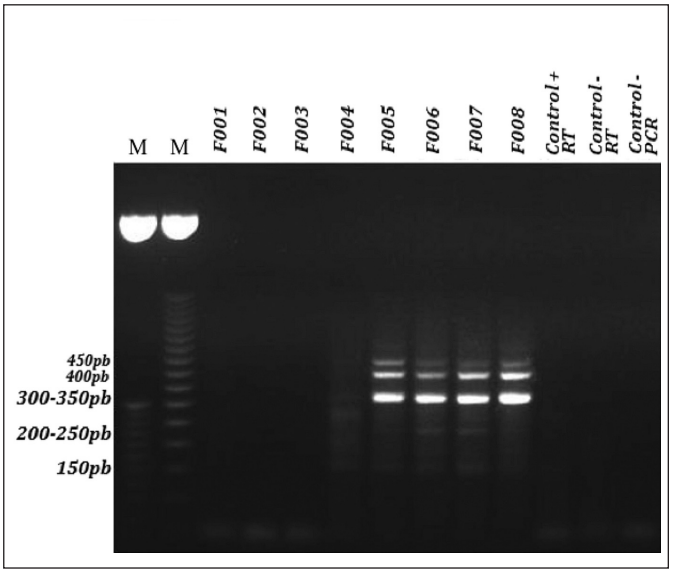

FIGURE 3. Agarose gel electrophoresis of amplified products by RT-PCR obtained with API2-MALT1 (AS2) gene oligonucleotides in samples F005 to F008. M, molecular-weight markers (25 bp and 50 bp).

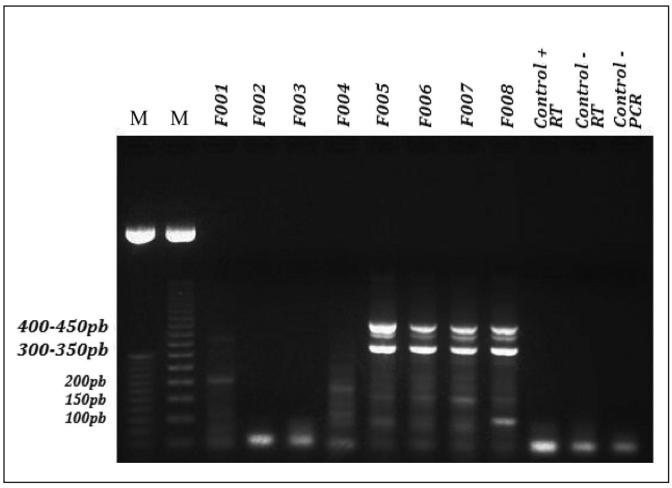

FIGURE 4. Agarose gel electrophoresis of amplified products by RT-PCR obtained with API2-MALT1 (AS3) gene oligonucleotides in samples F005 to F008. M, molecular-weight markers (25 bp and 50 bp).

DISCUSSION

Translocation t(11;18)(q21;q21) is the most frequently observed chromosomal aberration in GML[22]. This study demonstrated, by the first time in Brazil, translocation in 4/7 (57%) patients with low-grade GML, characterized by bands in agarose gel with about 300-350 and 400-450 bp, and its absence in a case of GML with a high grade component (patient F004). These findings are consistent with the literature, where t(11;18)(q21;q21) is found in 14 to 60% of GML cases, with a higher prevalence in the elderly population and in tumors at earlier stages[18, 21, 22, 29, 30]. It is worth mentioning that the average age in our study was 57 and all had tumor involvement restricted to the mucosa or submucosa.

HP was eradicated in all seven patients with proved infection, with histological tumor regression in 5/7 (71%) cases of low-grade GML. Absence of translocation in patients F001, F002 and F003 coincided with a favorable clinical course with complete tumor remission following bacteria eradication. Among the four patients with t(11;18)(q21;q21), two had histological tumor regression following bacteria eradication and the two remaining patients went on to need further cancer treatment.

It is now well established that HP eradication solely is able to induce the GML regression in up to 75% of cases[37]. However, the tumor regression process is slow, and can take up to 24 months[9, 10]. Findings showing that the presence of translocation t(11;18)(q21;q21) is predictive of an absence of tumor regression just by eradicating HP may significantly contribute to the decision on the best treatment to be applied, besides promoting reduction of medical care costs of following up on these patients[7, 19, 34]. Despite being a small sample, for now this study corroborates such findings, considering that half the translocation patients had no tumor regression following bacteria eradication. However, further studies should be conducted with a larger number of patients to

have more consistent results. Recent review has shown tumor remission rates of only 22% [95% condidence interval (CI): 3-41.4] in patients with such translocation[37].

Our molecular findings are compatible with the PCR products described in the literature, which highlight fragments of 67 bp and/or 340 bp for AS2 and fragments of 73, 100 and/or 409 bp for AS3[18]. The bands suggest that the fusion transcript API2-MALT1 found in the four positive patients is characteristic of the fusion between the exon E7 of the AP12 gene with the exon E5 of the MALT1 gene (Figure 5), corroborating the findings in the study by Liu et al. with a similar band pattern[18].

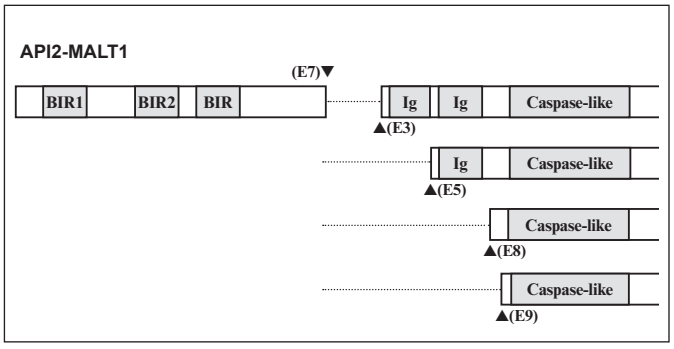

FIGURE 5. The most frequent AP12-MALT1 fusion products (adapted from ref. 18)

The Ig-like domain in the API2-MALT1 transcript resulting from t(11;18)(q21;q21), characterized by a band with a molecular weight of approximately 400 bp, relates directly with more aggressive forms of lymphoma and non-regression of GML following a positive response to anti-HP treatment[15]. The other bands suggest unspecific amplifications and/or alternative splices in the mRNA synthesis stage, due to suppression or deletion of one or more MALT1 gene exons, and it is quite likely that the API2-MALT1 (AS3) product amplifications from 100 to 250 bp are alternative splices from the transcript of the fusion between the genes[18]. The consequences of the alternative splices described in the literature are not yet clear. So far there are no records described regarding different sized bands from those mentioned. New information may be obtained from the direct sequencing of the transcripts, verification of similarity with some part of the other genes synthesized together, characterization of the alternative splices and a more detailed study of splicing points.

In conclusion, the investigation of the t(11;18)(q21;q21) in patients with GML is now a well established conduct, particularly in the early stages of the tumor. RT-PCR proved to be reproducible, feasible and efficient in detecting this genetic rearrangement, with an estimated cost of around US$ 400, and can be carried out in 3 to 5 days in routine molecular biology laboratories. The study of the t(11;18)(q21;q21) may also contribute significantly towards a better understanding about the pathogenicity of this disease, and the early detection of such translocation can be very helpful for better targeting the therapy to be applied to GML patients.

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Lima KS, Albuquerque W, Arantes VN, Drummond-Lage AP, Coelho LGV. *Helicobacter pylori* e presença de translocação t(11;18)(q21;q21) em pacientes portadores de linfoma MALT gástrico. Arq Gastroenterol. 2014,51(2):84-9.

RESUMO - ***Contexto*** - A patogênese do linfoma MALT (tecido linfoide associado à mucosa) gástrico, também conhecido como linfoma de zona marginal de células B, está claramente associada à gastrite por infecção pelo *Helicobacter pylori* e, a maioria desses tumores pode ser curada apenas com a erradicação da bactéria. A presença da translocação t(11;18)(q21;q21) tem sido identificada como a anomalia citogenética mais comum do linfoma MALT gástrico e sua presença pode prever uma menor taxa de resposta ao tratamento anti-*H pylori.* ***Objetivo -*** Estudo da translocação genética t(11;18)(q21;q21) e seu impacto na evolução clínica de pacientes portadores de linfoma MALT gástrico de baixo grau. ***Métodos*** - Uma série consecutiva de oito pacientes com linfoma MALT gástrico foi submetida à endoscopia digestiva, ultra-sonografia endoscópica, exame histopatológico, pesquisa do *H pylori* e metodologia rotineira de transcrição reversa seguida de reação em cadeia da polimerase (RT-PCR) utilizando primers específicos para API2-MALT1. Todos os pacientes receberam tratamento anti-*H pylori* e retratamento, quando necessário. Após a erradicação, exames endoscópicos foram realizados a cada 3-6 meses durante 2 anos para acompanhamento da evolução do tumor. ***Resultados -*** Foram estudados oito pacientes (seis mulheres, idade média: 57 anos). Todos apresentavam à ecoendoscopia envolvimento tumoral restrito à mucosa ou submucosa com aparência endoscópica variável. A histologia mostrou que sete pacientes tinham linfoma MALT gástrico de baixo grau e um de alto grau. A erradicação do *H pylori* foi obtida em todos os pacientes, embora a bactéria não tenha sido identificada em um deles. Foi observada regressão histológica do tumor em 5/7 (71%) dos pacientes com linfoma MALT gástrico de baixo grau. A presença de t(11;18)(q21;q21) foi identificada em quatro (57%) destes pacientes; entre eles, dois apresentaram regressão histológica do tumor após erradicação do *H pylori* e os outros dois não apresentaram remissão do tumor, sendo encaminhados para tratamento oncológico suplementar. ***Conclusão -*** A técnica de RT-PCR se mostrou reprodutível, factível e eficaz para detecção da t(11;18)(q21;q21), podendo ser realizada em poucos dias, em laboratórios de biologia molecular de rotina. A pesquisa da t(11;18)(q21;q21) em pacientes portadores de linfoma MALT gástrico é atualmente uma conduta bem estabelecida. A detecção precoce de tal translocação pode contribuir significativamente para a melhor definição da terapêutica a ser implementada aos portadores de linfoma MALT gástrico.

DESCRITORES - Linfoma de zona marginal tipo células B. *Helicobacter pylori*. Translocação genética. Transcrição reversa. Reação em cadeia da polimerase.

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