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SUMMARY

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SECTIONS

ORIGINAL ARTICLES

REVIEW ARTICLES

Undergraduate research in medical education

Iniciação científica na graduação médica

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Constant updating of scientific health knowledge is a great challenge for medical students, and a small part of them choose to pursue their academic career as researchers. However, satisfactorily mastering the scientific method is essential to develop the competency of critical thinking to assess new knowledge and emerging health technologies. Thus, it is important that, during medical undergraduate training, strategies are developed to awaken the vocation of students to become critically capable of analyzing scientific and technological knowledge, in order to contribute to the development of the country.[1,2]

Training activities in scientific research have been included as an integral part of the medical education curriculum in several countries.[3-8] In Brazil, Scientific Initiation Programs (ScIPs) have been the main strategy adopted with the goal of encouraging the scientific training of students through their participation in research projects, awakening the scientific vocation and encouraging new talent for research. These programs are widely offered in Brazilian higher education institutions, especially after the creation of the Institutional Scholarship Program under the responsibility of the National Council for Scientific and Technological Development (PIBIC/CNPq) in 1988.[3-8]

Most of the studies on the impact of ScIPs on the students' academic trajectory assessed their admission and performance in graduate programs. In fact, it has been observed that students who work in scientific research projects during their undergraduate training are more likely to pursue their master's and doctorate degrees, finishing these programs faster and with better academic performance than students who did not participate in research activities during medical school. In addition, studies indicate that although ScIP alumni do not necessarily become researchers, they have demonstrated greater communication and leadership skills, as well as teamwork in their professional activities.[9-21]

The interest of students in scientific activities has increased in medical courses. A study carried out in sixth year students from six Brazilian medical schools showed that only 7% had no interest in research.[12] Also, studies in Brazil and in other countries have shown that the main reasons that lead to participation in ScIPs are to improve the curriculum, learn the scientific method and present research results in scientific meetings and journals.[3-6,11,22-27]

Advisor-advisee interaction seems to be one of the most valuable experiences provided by the ScIP.[8] Accessibility, referral by other students, and scientific knowledge are the most cited reasons for choosing an advisor. The first two factors are directly related to the student's perception of a possible positive relationship with the advisor, which shows the importance of this aspect for the success of scientific initiation projects.[6,23,24] Moreover, the lack of integration between advisor and advisee has been pointed out as the main factor associated with lack of motivation and withdrawal of students from scientific initiation activities.[6,23]

In addition, considering the important role that ScIPs have been playing in the Brazilian medical education, it is important to know the main factors that can restrict access, so that strategies to improve institutional ScIPs are developed. One of these factors refers to the lack of

spare time for research activities, which has been pointed out by students as the main difficulty in developing research projects.[6,22] In this context, some institutions have acted to make scientific initiation a curricular activity, so that the student can have fixed hours reserved to research projects.[6,8,11,28-30]

Finding advisors that meet the students' expectations has also been pointed out as a factor that restricts student access to scientific initiation activities, since, in order to participate in ScIPs, students must have an advisor developing research projects on topics of their interest. Limiting factors may be associated with other aspects, such as deficits in physical infrastructure, lack of financial resources, lack of student motivation and lack of motivation or lack of qualification of the teaching staff, and it is therefore important that institutions adopt measures to encourage faculty members to conduct research activities including undergraduate students.[2,6,8,31]

Conflict of interest

The authors declare no conflict of interest.

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Prostate cancer – Therapy with radium-223

Câncer de próstata – Terapia com rádio-223

Authorship: Brazilian Society of Nuclear Medicine

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize procedures to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

Description of the evidence collection method

This guideline followed the standard of a systematic review with evidence retrieval based on evidence-based medicine (EBM), so that clinical experience is integrated with the ability to critically analyze and apply scientific information rationally, thus improving the quality of medical care.

We used the structured mode of formulating questions synthesized by the acronym PICO, where P stands for patients with prostate cancer, I stands for indicator, i.e., radium-223, and O stands for outcome, which is benefit.

Based on the structured question, we identified the descriptors that formed the basis of the search for evidence in the databases: Medline-Pubmed. Ninety-nine (99) studies were retrieved and, after applying the eligibility criteria (inclusion and exclusion), 13 were selected to answer the clinical question (Annex I).

Clinical question

What is the benefit of radium-223 therapy in prostate cancer?

Grades of recommendation and levels of evidence

- A: Experimental or observational studies of higher consistency.
- B: Experimental or observational studies of lower consistency.
- C: Cases reports / non-controlled studies.
- D: Opinion without critical evaluation, based on consensus, physiological studies or animal models.

Objective

The aim of this guideline is to estimate the benefit of radium-223 chloride therapy (Ra223) in patients with prostate cancer resistant to castration and bone metastases.

Introduction

Ra223 has been approved by the Brazilian Health Surveillance Agency (Anvisa) to be used as a drug in the treatment of patients with bone metastases from metastatic castration-resistant prostate cancer (mCRPC) without known visceral metastases.[1]

This treatment is indicated when patients with mCRPC have blastic bone metastases in an attempt to increase survival, but it is also applied in these conditions when patients have symptoms, such as pain (but not exclusively).

Ra223 is a low-range (< 100 µm), high-energy (27.4 MeV) alpha particle emitter, similar to calcium, which is directed to areas where bone remodeling is increased, which occurs in sclerotic bone metastases of prostate cancer. The high energy of alpha particles causes the DNA to break down in the metastatic cells (cytotoxic effect), with low damage in the adjacent cells due to their low reach (< 10 cells).[2]

Data extraction

Survival

After evaluating 64 mCRPC patients eligible for radiation therapy due to bone pain, with mean follow-up time of 18 months, there was greater survival among patients treated with Ra223 (50 kBq/kg dosage) compared to a placebo. Median survival was 65.3 versus 46.4 weeks, respectively, significantly higher for Ra223 (50 kBq/kg dosage) (p=0.006) compared to a placebo (HR=2.12, 95CI 1.12-3.98; p=0.020), indicating a higher risk of death for the placebo group.[3] (**A**) After 2 years of follow-up of these patients, the median survival of the Ra223-treated group was 71 weeks versus 46.4 weeks in the placebo group (HR=0.445, 95CI 0.232-0.851).[4] (**A**)

In the largest phase III multicenter controlled randomized trial evaluating 921 mCRPC patients, 614 received six injections of Ra223 (50 kBq/Kg dosage) and 307 received placebo injections. Those patients treated with Ra223 had greater survival compared to those treated with placebo, with a median survival of 14.9 versus 11.3 months. The absolute increase in survival was 10.5 months (95CI 3.3-17.7). In this case, it is necessary to treat nine patients to have an increase in survival of 3.6 months (on average) in one patient.[5] (**A**)

When this same population was stratified according to prior use of docetaxel, Ra223 continued to show an increase in survival, both in the group of prior use (HR=0.70, 95CI 0.56-0.88; p=0.002) compared to the group that had never used docetaxel (HR=0.69, 95CI 0.52-0.92; p=0.01).[6] (**A**)

Bone event

In an initial study with 64 mCRPC patients, there was longer time until the first bone event in the Ra223-treated group compared to the placebo group (14 weeks versus 11 weeks) (HR=1.75, 95CI 0.96-3.19; p=0.065). However, there was no significant difference in the number of bone events (p=0.625).[3] (**B**)

In the large ALSYMPCA study, evaluating 921 patients with mCRPC, patients treated with Ra223 had longer time until the first bone event compared to the placebo group. Median time until the first bone event was 15.6 months with Ra223 versus 9.8 months with a placebo (HR=0.66, 95CI 0.52-0.83; p<0.001).[5] (**A**)

The data from the above study were further analyzed, focusing exclusively on the skeletal events of the mCRPC population.[7] (**A**) The authors demonstrated that symptomatic skeletal events occurred in 33% of patients in the Ra223 group and 38% in the placebo group. The Ra223 group required less use of radiotherapy to treat pain (HR=0.67, 95CI 0.53-0.85) and presented a lower spinal compression rate (HR=0.52, 95CI 0.29-0.93). Despite this, the use of Ra223 did not significantly reduce the risk of occurrence of bone events (HR=0.62, 95CI 0.35-1.09) nor the risk of surgical interventions (HR=0.72, 95CI 0.28-1.82).[7] (**A**)

Another study stratified the population described above according to prior use of docetaxel or not. 352 patients who used docetaxel prior to Ra223 were evaluated versus 262 patients who used only Ra223. There was a reduction in time until the first bone event only in the group of patients taking Ra223 who had previously used docetaxel (p=0.0009).[6] (**A**)

Dose toxicity and safety

In the initial randomized controlled multicenter phase II trial of 64 mCRPC, with patients divided into Ra223 and placebo groups, there was no significant difference in hematological toxicity. In addition, no patient discontinued treatment for this reason.[3] (**A**)

The use of different doses of Ra223 (5 kBq/kg in 26 patients; 25 kBq/kg in 25 patients; 50 kBq/kg in 25 patients; 100 kBq/kg in 24 patients) in 100 mCRPC patients showed no difference in adverse effects among the groups analyzed, and 97% of the patients reported at least one adverse effect. Adverse effects included nausea, fatigue, vomiting, diarrhea, constipation, bone pain, urinary tract infection and peripheral edema.[8] (**A**)

Then, another study with a slightly larger casuistry (122 patients) was conducted to evaluate the efficacy and safety of different dosages of Ra223. The doses administered were 25 kBq/kg in 41 patients, 50 kBq/kg in 39 patients and 80 kBq/kg in 42 patients, each of them undergoing a protocol of three applications every 4 weeks. The study demonstrated that dosages up to 80 kBq/Kg are safe. Ninety-two per cent (92%) of the patients had at least one adverse effect: diarrhea (21%), nausea (16%) and anemia (14%). There were no differences among the groups regarding survival, bone events, pain reduction or hematological events.[9] (**A**)

In the ALSYMPCA study (N=921), the overall number of adverse and hematological effects was lower in patients treated with Ra223 (93%) compared to placebo (96%), with 47% serious events in the first and 60% in the latter group. The number of grade 3 or 4 adverse hematological effects was not significantly higher in the group treated with Ra223.[5] (**A**)

Subgroup analysis of ALSYMPCA patients stratified by prior docetaxel use showed that the incidence of grade 3 or 4 anemia and neutropenia was similar between Ra223 and placebo, regardless of previous use of docetaxel.[6] (**A**)

However, previous use of docetaxel led to a greater number of hematological adverse events in both Ra223 and placebo patients. However, grade 3 and 4 myelosuppression rates were low, with differences only in thrombocytopenia rates. In addition, previous use of docetaxel did not influence the number of non-hematological events.[6] (**A**)

The main non-hematological adverse events reported in the studies were diarrhea, constipation, vomiting, nausea, fatigue, bone pain and peripheral edema.[3,5,6,9,10] (**A**)

Markers

Evaluating the different dosages of Ra223 (25, 50 and 80 kBq/kg) in a total of 122 patients, there was a better alkaline phosphatase (AP) and prostate specific antigen (PSA) response in the groups receiving the highest doses (50 and 80 kBq/kg). No patient in the 25 kBq/kg group achieved a 50% reduction in PSA.[9] (**A**)

In the ALSYMPCA study, there was a significant decline in AP and a longer time interval for elevation of this marker in patients treated with Ra223 compared to placebo. There was also a significant reduction of PSA in the Ra223 (16%) versus placebo (6%) group, in addition to a longer time interval to raise this parameter.[5] (**A**)

In both studies described above, the AP and PSA values were analyzed after 12 weeks and the reduction was considered significant if greater than 30% of the initial value. This longer time to increase AP and PSA also occurred in the Ra223 group compared to placebo, regardless of previous docetaxel use ($p<0.05$).[6] (**A**)

Improvement of pain and quality of life

A study with 100 mCRPC patients aimed at evaluating different single doses of Ra223 (5, 25, 50 and 100 kBq/kg) and had as primary outcome improvement of pain within 16 weeks after treatment. In this study, the groups receiving the highest Ra223 dosages (50 and 100 KBq/Kg) required less of other forms of analgesia for pain control than the groups receiving lower Ra223 dosages (5 and 25 kBq/kg).[8] (**A**)

In the analysis of pain reduction alone among the different doses of Ra223 (25, 50 and 80 kBq/kg) given to 122 patients, there was a tendency for better results using 50 kBq/kg.[9] (**A**) Despite the dose-response effect on pain reduction, there is no such analysis compared to placebo.

A subgroup analysis of patients from the ALSYMPCA[5] study evaluated the improvement of quality of life. More patients treated with Ra223 showed improvement in quality of life assessment tests compared to patients treated with placebo (29.2% versus 18.5%; $p=0.004$).[10] (**A**)

Hospitalization rate

Another study evaluated the hospitalization rate within the 12 months following treatment with Ra223 compared to placebo-treated patients. Patients receiving Ra223 had a lower hospitalization rate than those treated with placebo (37% and 45.5%, respectively), regardless of whether a skeletal event occurred. In addition, the number of days of hospitalization in the Ra223 group was lower than in the placebo group (4.4 versus 6.6 days; $p=0.004$).[11] (**A**)

Evidence summary

We were able to perform a meta-analysis of two outcomes studied: survival and bone event. The other outcomes could not be investigated by meta-analysis due to the lack of necessary data or use of the same population in different studies.

Survival

Two studies[4,5] (**A**) totaling 985 patients (647 in the Ra223 group and 338 in the placebo group) were included in this analysis. In this comparison, at 0% heterogeneity, there was a 10% decline in mortality (95CI 4-16) in favor of Ra223 treatment.[12] (**A**) Thus, 10 patients need to be treated to avoid one death compared to no treatment (Figure 1).

Bone events

Two studies were included in the analysis[3,7] (**A**) totaling 985 patients (647 treated with radium-223 and 338 with a placebo). In this comparison there is a non-significant reduction in the risk of bone events of 5% (95CI -1–11) in favor of treatment with Ra223 and heterogeneity is 0%. This means that there is no significant difference ($p<0.05$) in the risk of bone events when comparing treatment and non-treatment with radio-223 (Figure 2).

Recommendation

Ra223 is effective for the treatment of patients with mCRPC, with a reduction in mortality of 10% and an increase in mean survival over 3 months. There is no reduction in the number of bone events in these patients and no improvement in pain was observed except for the dose-response aspect.

Other benefits have been demonstrated, such as improved quality of life, increased time to skeletal events, reduced hospitalization and effect on markers such as PSA and AP.

The most common adverse events are both hematologic (anemia, neutropenia and thrombocytopenia) and non-hematological (diarrhea, constipation, vomiting, nausea, fatigue, bone pain and peripheral edema).

Study or subgroup	Radio 223 Events	Total	Placebo Events	Total	Weight	Risk difference M-H, Fixed, 95CI
Nilsson 2007	0	0	0	0	0.0%	-0.74 [-0.90, 0.58]
Nilsson 2013	23	33	27	31	7.2%	-0.17 [-0.37, 0.02]
Parker 2013	333	614	195	307	92.8%	-0.09 [-0.16, -0.03]
Total (95CI)		**647**		**338**	**100.0%**	**-0.10 [-0.16, -0.04]**
Total events	356		222			
Heterogeneity Chi² = 0.60, df = 1 (p=0.44); I² = 0%						
Test for overall effect: Z = 3.05 (p=0.002)						

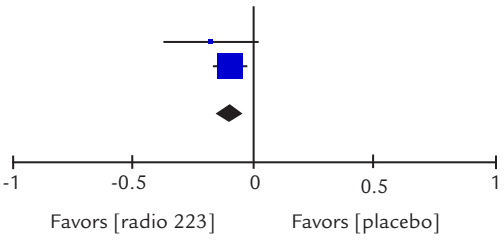

FIGURE 1 Summary of the studies comparing the use of Ra223 versus placebo in the treatment of mCRPC regarding the outcome of increased survival.

Study or subgroup	Radio 223 Events	Total	Placebo Events	Total	Weight	Risk difference M-H, Fixed, 95CI
Nilsson 2007	17	33	18	31	7.2%	-0.07 [-0.31, 0.18]
Sartor 2014	200	614	116	307	92.8%	-0.05 [-0.11, 0.02]
Total (95CI)		**647**		**338**	**100.0%**	**-0.05 [-0.11, 0.01]**
Total events	219		134			
Heterogeneity Chi² = 0.02, df = 1 (p=0.90); I² = 0%						
Test for overall effect: Z = 1.55 (p=0.12)						

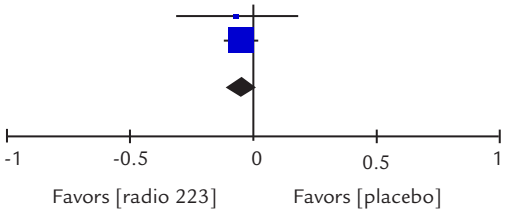

FIGURE 2 Summary of the studies comparing the use of Ra223 versus placebo in the treatment of mCRPC regarding the outcome of number of bone events.

Treatment with six doses of 55 kBq/Kg of intravenous Ra223 injections every 30 days is recommended for patients with mCRPC and bone metastases.

Discussion and perspectives

At the moment only five other medications, in addition to Ra223, which produce a demonstrated increase in survival in patients with mCRPC (docetaxel, cabazitaxel, abiraterone, enzalutamide, sipuleucel-T) are available. In view of this scenario, Ra223 stands out as a treatment with few contraindications and acceptable adverse effects, and an excellent option for mCRPC patients.

Although the studies presented here use a dose of 50 kBq/kg, the commercial dose was adjusted to 55 kBq/kg to meet the standardization criteria.[13] (**D**)

Only one study carried out re-treatment with Ra223 in mCRPC patients.[14] (**B**) Although it is a possibility, since the study showed safety, we do not recommend repeating the treatment until further studies are performed.

Studies are being conducted to validate the concomitant use of Ra223 with other therapies. We highlight the combination of Ra223 treatment with enzalutamide (phase III studies), abiraterone (phase II: NCT02097303), denosumab (phase II: NCT02582749) and radiotherapy (phase II: NCT02484339). In addition, studies in asymptomatic patients are being performed (NCT03002220).

Ra223 is also being studied to treat other diseases such as osteosarcoma (NCT01833520), multiple myeloma (NCT02928029) and breast cancer (phase II: NCT02258451).

As soon as these studies are available, we will update this guideline.

Conflict of interest

The authors state that there is no conflict of interest regarding this review.

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Annex I

Structured question

- **P** – Patients with prostate cancer resistant to castration and bone metastases.
- **I** – Intravenous therapy with radium-223.
- **C** – Placebo.
- **O** – Benefit or harm.

Search strategy

PubMed-Medline

- #1 - (Prostate Neoplasms OR Prostate Neoplasm OR Prostatic Neoplasm OR Prostate Cancer OR Prostate Cancers OR Prostatic Cancer OR Prostatic Cancers)
- #2 - (radium OR Xofigo OR Ra 223)
- #3 - random*
- 1st RETRIEVAL = #1 AND #2 AND #3 = 99

Articles retrieved

Ninety-nine (99) articles were retrieved. Of these, 19 were selected by title and 11 by summary. After a critical analysis by three nuclear physicians, 13 studies were selected, using as inclusion criteria randomized clinical trials, greater strength of evidence and outcomes pertinent to the clinical doubt in question. The reason for excluding the other texts was that they were not randomized studies.

The scientific database consulted was Medline via Pubmed. A manual search was performed based on references of the reviews (narrative or systematic), as well as the selected studies.

Inclusion criteria for selected studies

Increased survival was the main outcome analyzed in this guideline; however, during its elaboration, it was possible to evaluate other outcomes, which are also presented.

According to study design

The studies included in this guideline were classified according to the Jadad score.[15] According to this classification, studies with Jadad less than three are inconsistent, while those with a score greater than three are considered consistent.

Language

We included studies available in Portuguese, English or Spanish.

According to publication

Only full-text studies were considered for critical assessment.

Exposure of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and/or harm and controversies will be defined in a specific manner, whenever possible.

Recommendation

The recommendations will be elaborated by the authors of the review, with the initial characteristic of synthesis of the evidence, and later validated by all the authors who participate in the elaboration of this Guideline.

The grade of recommendation stems directly from the available strength of included studies, according to the Oxford scale,[16] and the GRADE system.[17]

Concomitant testicular infection by Zika virus and *Schistosoma mansoni*

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Dear Editor, the publication on "Concomitant testicular infection by Zika virus and *Schistosoma mansoni*" is very interesting.[1] Alves et al. noted that "In endemic areas, orchiepididymis by *Schistosoma* should be investigate to avoid unnecessary surgeries. This patient was also infected with Zika virus."[1] Indeed, this report is a good case study showing that the Zika virus can concurrently occur with any other infections. In tropical endemic countries, coinfection with Zika virus and other tropical disease is not uncommon and this might result in increased difficulty in diagnosis. The classic example is the concurrent infection with Zika and other mosquito-borne virus.[2] In the present case, concurrent Zika virus infection and schistosomiasis does occur, but it seems to be only a coincidence without any significant clinical observation. Nevertheless, since there are some reports on the effect of Zika virus on testis in animal models,[3,4] the long term follow-up of the case regarding fertility might provide some useful clinical data.

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Gaucher's disease in a patient presenting with hip and abdominal pain

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Summary

Gaucher's disease is characterized by glucocerebroside accumulation in the cells of the reticuloendothelial system. There are three subtypes. The most common is type 1, known as the non-neuropathic form. Pancytopenia, hepatosplenomegaly and bone lesions occur as a result of glucocerebroside accumulation in the liver, lung, spleen and bone marrow in these patients. Findings associated with liver, spleen or bone involvement may be seen at radiological analysis. Improvement in extraskeletal system findings is seen with enzyme replacement therapy. Support therapy is added in patients developing infection, anemia or pain. We describe a case of hepatosplenomegaly, splenic infarction, splenic nodules and femur fracture determined at radiological imaging in a patient under monitoring due to Gaucher's disease.

Keywords: Gaucher's disease, tomography, sphingolipidoses.

Introduction

Gaucher's disease is an autosomal recessive lipid storage disorder. The beta-glucocerebrosidase enzyme is coded in chromosome 1q21, and the disease results from mutation in this gene. In this lipid storage disease, glucocerebroside is stored in the cells of the reticuloendothelial systems as a result of beta-glucocerebrosidase deficiency.[1] There are three subtypes of Gaucher's disease, the principal determining characteristic of which is the presence or absence of neurological involvement. Type 1 is the chronic non-neuropathic form, seen in the adult age group. Type 2 is the acute neuropathic form, which is characterized by progressive neurological involvement in addition to hepatosplenomegaly.[2] Type 3, the subacute neuropathic form, is seen in children aged between 2 and 6, and involves mild neurological findings and hepatosplenomegaly. Beta-glucocerebrosidase enzyme deficiency is the common feature of all three subtypes.[1,2] Glucocerebroside particularly accumulates in the liver, lung, spleen and bone marrow. The clinical manifestations of this accumulation are pancytopenia, hepatosplenomegaly, pulmonary involvement, bone lesions and renal injury.[3]

Case report

A 56-year-old woman presented to our hospital with hematuria during the previous week and persistent pain in the right hip region during the previous 6 months. She stated that the hip pain began after a minor trauma and persisted despite use of analgesics. Her medical history revealed that she had been diagnosed with Gaucher's disease at the age of 38 and had two siblings with the same condition. She described having anemia since her youth, and reported that bleeding occurred with mild traumas, causing occasional hematuria. Radiography and magnetic resonance imaging (MRI) of the right hip joint revealed edema and fracture in the femoral neck, and decreased convexity, irregularity and osteonecrosis in the femoral head (Figures 1 and 2). Inhomogeneity in bone marrow and focal hypointense areas were also observed in the pelvic bones on T1 weighted series. Abdominal computerized tomography (CT) findings were hepatosplenomegaly, splenic infarction and hypodense nodules in the splenic parenchyma (Figure 3). At laboratory examination, hemoglobin was 9.6 mg/dL and platelet count, 16,000. The platelet count failed to increase sufficiently despite transfusion, fracture surgery was postponed at the patient's request, and she was referred to the internal diseases clinic.

Discussion

Gaucher's disease was first described by Gaucher in 1882. This lipid storage disease results from mutation in the

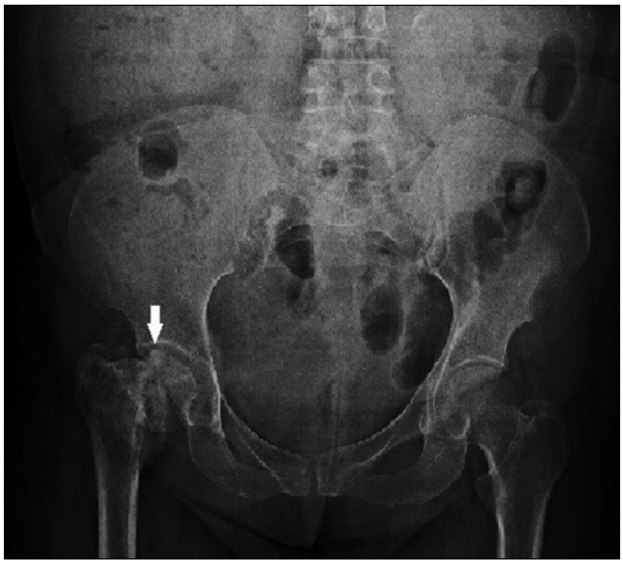

FIGURE 1 Decreased convexity, irregularity and sclerotic foci in the right femoral head.

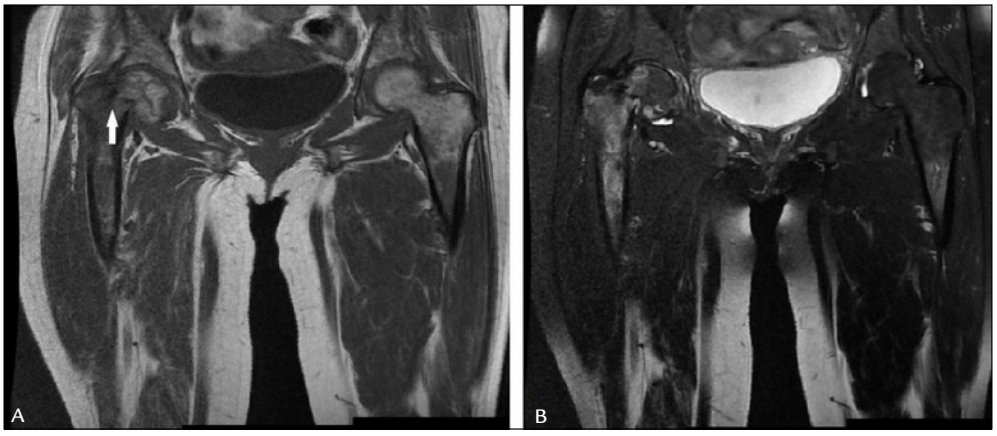

FIGURE 2 Fracture line (A) and bone marrow edema (B) in the femoral neck.

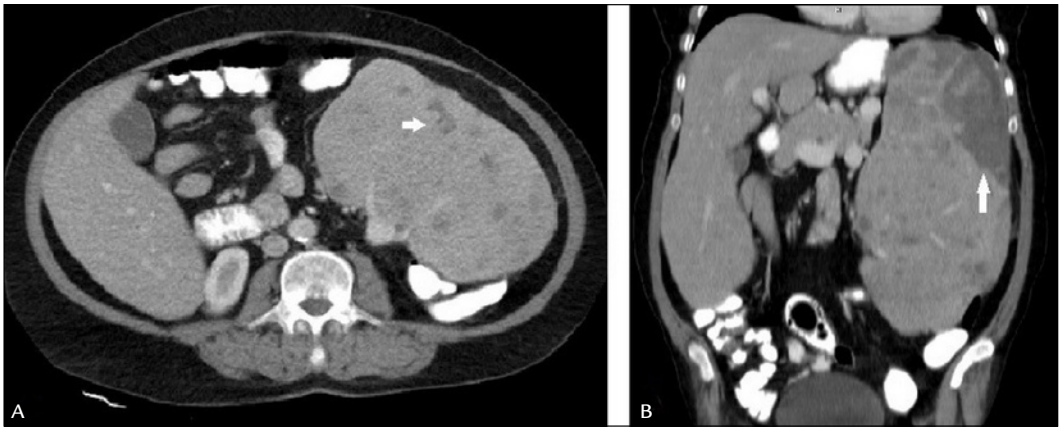

FIGURE 3 Hepatosplenomegaly, splenic nodules (A) and splenic infarct (B) at abdominal CT.

gene encoding the enzyme beta-glucocerebrosidase. It has been reported to be more common among Ashkenazi Jews.[2,4] Additionally, multiple myeloma, lymphoma, hepatocellular carcinoma and Parkinson disease are more common in Gaucher patients than in the normal population.[3] Beta-glucocerebrosidase enzyme activity is impaired as a result of mutations, and glucocerebroside accumulation occurs in the reticuloendothelial system.[1,3] The most commonly seen subtype is type 1, characterized by hepatosplenomegaly, hematological findings and bone diseases.[2] Symptoms commonly occur in the 3rd decade of life in this chronic type. Prognosis is poorer in symptomatic patients in the pediatric age group.[2]

Abdominal pain associated with hepatosplenomegaly is frequently seen among the clinical findings. Infarctions, nodules, portal hypertension and cirrhosis may occur in the liver. Hepatocyte damage, fibrosis around Gaucher cells and collagen bands appear in the liver. Hepatomegaly is present in almost all patients, but impairment of liver functions is rare.[4,5] In our case, alkaline phosphatase, aspartate aminotransferase and gamma glutamyl transferase enzymes were slightly elevated (110 U/L, 37.4 U/L and 51 U/L, respectively). Increased biliary excretion of glucosylceramide, hepatic injury and gallstone formation also occur in these patients.[1] Increasing hemolysis is implicated in the formation of gallstones. However, no gallstone or biliary duct pathology were present in our case.

Splenomegaly is frequently the earliest finding determined. Care must be taken in terms of complications such as bleeding resulting from splenic involvement, increased frequency of infection, hypersplenism, splenic infarct and fibrosis. Rapid growth in the spleen occurs mostly in childhood. If rapid splenic growth occurs in adulthood, hematological malignancy and autoimmune hemolytic anemia must be investigated.[6]

Splenectomy is currently used in the treatment of a limited number of patients. This is due to the possibility of post-splenectomy complications and cell infiltration in bone marrow.[7] Hypersplenism and bone marrow infiltration play a role in the development of pancytopenia in type 1 Gaucher's disease patients. In our case, the long axis of the spleen was 260 mm, and splenic infarct and intraparenchymal nodular involvement were present. Other hematological findings were anemia persisting for many years, a history of bleeding with minor traumas and hematuria.

The pathophysiology of bone involvement is not fully known, although bone and bone marrow infiltration of Gaucher cells is implicated. The most commonly affected bone is the femur, and the most common lesions are osteopenia, osteonecrosis, osteosclerosis and bone infarcts.[6] Chronic mild joint pain or severe pain mimicking sickle cell anemia may occur. Infarcts cause an acutely painful condition known as bone crisis by increasing intraosseous pressure. Functional imbalances of osteoblasts and osteoclasts impair balance between bone formation and destruction.[8] The most common radiological findings in bone involvement are Erlenmeyer flask deformity, osteopenia, avascular necrosis and infarction. The radiological appearance of fibrous proliferation and trabecular resorption secondary to infiltration takes the form of cortical thinning, scalloping and radiolucency. Other skeletal findings are compression fracture in the vertebral body, secondary osteoarthritis and pathological fracture.[8,9]

Use of enzyme replacement therapy and support therapy apply in Gaucher's disease. Improvement has been reported in extraskeletal findings and hematological parameters with enzyme replacement therapy.[7] Support therapy may include antibiotherapy for infections, iron formulations for anemia, analgesic for bone pathologies and surgical procedures. Use of splenectomy is limited and it is generally employed in cases of massive infarct or severe pancytopenia.[10]

Conflict of interest

The authors declare no conflict of interest.

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Cogan's syndrome – A rare aortitis, difficult to diagnose but with therapeutic potential

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Summary

The inflammation of aortic wall, named aortitis, is a rare condition that can be caused by a number of pathologies, mainly inflammatory or infectious in nature. In this context, the occurrence of combined audiovestibular and/or ocular manifestations eventually led to the diagnosis of Cogan's syndrome, making it the rare case, but susceptible to adequate immunosuppressive treatment and satisfactory disease control.

Keywords: chest pain, aortitis, Cogan's syndrome.

Introduction

Inflammation of the aortic wall, called aortitis, is an infrequent clinical condition that manifests itself with systemic symptoms and may cause precordial pain.[1-4] One of the rheumatologic causes of aortitis is a rare disease called Cogan's syndrome.[5] With approximately 300 cases reported in the world, it has no clearly defined etiology, pathophysiology, diagnosis or treatment,[5] and affects from children to the elderly but mostly young adults (mean age 30 years).[5] The occurrence of vestibular and ophthalmologic symptoms associated with the condition aided in the diagnosis of the case described, which, despite being rare, may respond to adequate immunosuppressive treatment achieving satisfactory disease control.[6]

Case report

This is the case of a 63 year-old male patient, Caucasian, born and living in the city of São Paulo, who attended the emergency department with a complaint of severe precordial chest pain described as tightness and irradiated to the mandible 2 hours before admission to hospital, combined with sweating and dyspnea. The patient reported the repeated occurrence of the symptoms, although less intense, for more than ten years, and that four years ago the episodes began to intensify. He was admitted to another service a week before for the same reason, where he underwent coronary angiography, showing no coronary obstruction, and an echocardiogram, which revealed a slight dilatation of the ascending aorta. In addition, the patient reported bilateral hypoacusis for 10 years (progressing to deafness in the right ear), rotational vertigo for 8 years and hyperemia, pain and eye tearing for two years. At admission, he did not regularly use any medications. He denied having risk factors for coronary heart disease or other comorbidities.

At physical examination, the patient presented a regular general condition, he was sweating and pale, with a heart rate of 75 beats per minute, breathing normally, with peripheral arterial oxygen saturation of 97%, blood pressure of 130 x 80 mmHg in both arms, ictus not visible but palpable with fingertip in the normal position at the fourth intercostal space under the left midclavicular line, rhythmic heart sounds, no bruit heard and pulmonary auscultation with preserved vesicular murmur bilaterally without adventitious breath sounds. Pulse was heard bilaterally, wide and symmetrical. Jugular stasis was absent bilaterally at 45 degrees.

ECG results revealed sinus rhythm, without abnormalities suggestive of myocardial ischemia. At that moment, the

diagnostic hypothesis of possible acute aortic dissection was raised. The initial treatment choice was to administer intravenous metoprolol and sodium nitroprusside, followed by immediate thoracic and abdominal aortic angiotomography.

Aortic angiotomography showed ectasia of ascending aorta (maximum caliper of 4.1 cm), with no signs of dissection, and with slight parietal thickening of the ascending aorta, which remained with a small adjacent pericardial effusion, suggestive of aortitis (Figure 1).

From that moment, the inflammatory symptoms of probable aortitis began to be investigated. Transthoracic echocardiography showed grade 1 left ventricular diastolic dysfunction and discrete sinus and ascending aorta ectasia with preserved left ventricular systolic function. Laboratory tests revealed negative myocardial necrosis markers, leukocytosis (14,070 leukocytes/mm^3 without left shift), increased C-reactive protein (CRP) (245.97 mg/L) and increased erythrocyte sedimentation rate (ESR) (49). Rheumatologic markers (rheumatoid factor, antinuclear factor, complement fractions C3 and C4, and anti-neutrophil cytoplasmic antibodies), serology for hepatitis B, hepatitis C and HIV, syphilis and cultures (blood and urine) were requested. All results were within normal range.

The patient underwent positron emission tomography (PET) to confirm the hypothesis of aortitis, which revealed a moderate diffuse uptake in the aortic wall from its root to the middle portion of the aortic arch, as well as a discrete/moderate diffuse image with thickening of the pericardial sac and effusion in its interior (Figure 2). Nuclear magnetic resonance imaging was also performed and showed only pericardial thickening.

Until that moment, the patient still had recurrence of the precordial pain episodes, being initially medicated with common analgesics and opioids. However, based on the adjuvant clinical history of ophthalmologic and audiovestibular involvement and the complementary exams performed, we raised the diagnostic hypothesis of Cogan's syndrome.

Treatment with prednisone 1 mg/kg/day was started on day 3 of hospitalization. From that day on, the patient progressed with important clinical improvement and did not present new episodes of precordialgia.

The patient was discharged on day 7 of hospitalization and day 5 of corticoid use; he was asymptomatic and his CRP level was 48.92 mg/L. After two weeks, he returned for a follow-up visit still undergoing treatment with prednisone and remained asymptomatic, with CRP and ESR levels at 1.34 mg/L and 6, respectively.

Discussion

Chest pain, as in our patient, is a common symptom in the emergency unit and deserves special attention due to the numerous diagnostic possibilities. Well-indicated anamnesis, physical examination and complementary exams are fundamental for the differential diagnosis. The diagnosis of acute coronary syndrome was removed since the results of the patient's coronary angiography did not reveal coronary obstructions and the ECG had no signs of myocardial ischemia. Changes in angiotomography, performed for the investigation of acute aortic dissection, raised the diagnostic hypothesis of aortitis.

Aortitis is an uncommon clinical condition, defined by inflammation of the aortic wall.[1-4] It produces nonspecific symptoms such as fever, fatigue and weight loss, and may present with angina, aortic insufficiency and aortic dissection.[2] It can be caused by infectious and non-infectious diseases, the latter being more common.[1-3] Vasculitis, including giant cell arteritis and Takayasu's arteritis, are the most common causes of aortitis. Rarer conditions such as Cogan's syndrome are also mentioned in this context (Table 1).[1,2]

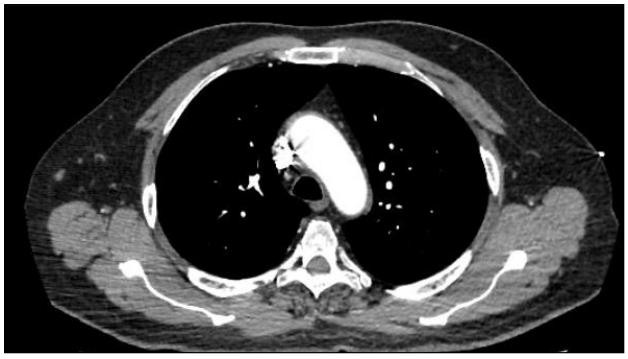

FIGURE 1 Angiotomography revealing parietal thickening in the ascending aorta.

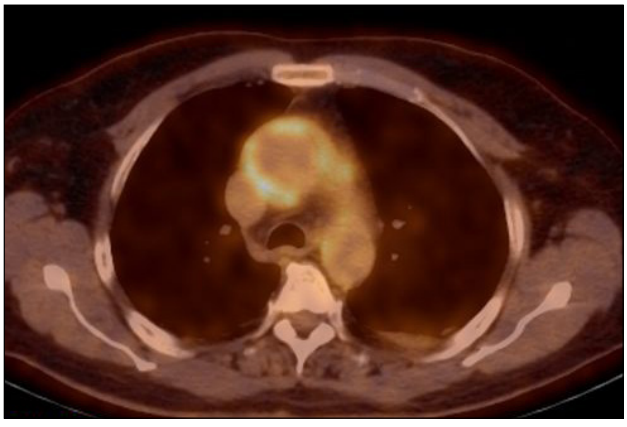

FIGURE 2 PET scan revealing moderate diffuse pattern capture in aortic walls.

TABLE 1 Main causes of aortitis according to etiology.

Non-infectious aortitis
Large vessel vasculitis
Giant cell arteritis
Takayasu's arteritis
Rheumatoid arthritis
Systemic lupus erythematosus
Spondyloarthropathies
• Ankylosing spondylitis
• Reiter's syndrome
Other types of vasculitis
ANCA-associated vasculitis
• Wegener's granulomatosis
• Polyarteritis nodosa
• Microscopic polyangiitis
Behçet's disease
Cogan's syndrome
Relapsing polychondritis
Sarcoidosis
Idiopathic
• Idiopathic isolated aortitis
• Chronic periaortitis
• Inflamed aortic aneurysm
Radiation-induced aortitis
Infectious aortitis
Bacteria
• Salmonella
• *Staphylococcus* spp
• *Streptococcus* spp
Syphilis
Mycobacteria
• Mycobacterium tuberculosis
Virus
• HIV

The diagnosis of aortitis is difficult due to the lack of specificity of symptoms and low clinical suspicion,[1] often obtained only with biopsy and histopathological analysis.[4] Laboratory tests, such as inflammatory activity markers (C-reactive protein and ESR), aid in diagnosis when high levels are present, but are non-specific.[2] Angiography has now been replaced by less invasive examinations, such as angiotomography and angioresonance.[2] Both play a key role, since they detect thickening of the aortic wall, dilation and stenosis. Recently, PET tomography has been shown to be an important examination because it demonstrates increased aortic wall metabolism through the uptake of fluoride-18-labeled fluorordesoxyglucose.[1,2,5] It has high sensitivity and specificity, reaching 92 and 100%, respectively.[1] It is useful not only to obtain a diagnosis, but also for follow-up, evaluating the response to treatment.[1,5] In our patient, the suspicion of aortitis arose due to the presence of parietal thickening in the ascending aorta at angiotomography. High CRP and ESR values corroborated our suspicion. The results of PET tomography, which demonstrated a moderate diffuse uptake of the aortic wall from its root to the middle portion of the aortic arch, were fundamental for the diagnosis of aortitis.

Defining the etiology of aortitis is not always an easy task, but it is essential for adequate treatment. It is based on epidemiological, clinical, laboratory and imaging data. General laboratory tests, such as blood count, electrolytes, renal function and liver function are part of the initial evaluation.[2] It is imperative to rule out an infectious cause by collecting samples for culture and targeted research if there is any clinical suspicion of infection.[2] The investigation of rheumatic diseases is also part of it, since they are the main causes of aortitis. Measuring rheumatologic markers such as rheumatoid factor, antinuclear factor, complement fractions (C3 and C4) and anti-neutrophil cytoplasmic antibodies is always indicated.[2] In our case, the clinical history was fundamental for the diagnostic suspicion of Cogan's syndrome. In addition to aortitis, the patient had a history of bilateral hypoacusis in the last 10 years (progressing to deafness in the right ear), rotational vertigo for 8 years, and hyperemia, pain and eye tearing for 2 years. After ruling out any infectious causes of aortitis and other more frequent rheumatic diseases, the hypothesis of Cogan's syndrome became more evident.

Cogan's syndrome is an extremely rare condition, with approximately 300 cases reported worldwide.[5] It does not have well-defined etiology, diagnosis or treatment. It is believed that autoimmune mechanisms are involved, but its pathophysiology remains unknown.[5] It affects from children to the elderly, mainly focusing on young adults (mean of 30 years).[5] It is characterized by ocular changes such as hyperemia, pain, tearing, photophobia and foreign body sensation, accompanied by audiovestibular manifestations similar to Ménière's disease, characterized by vertigo, tinnitus, nystagmus, nausea and hearing loss with a sensorineural pattern, which can lead to deafness in a short term.[6] The mean interval between ocular and auditory involvement is a few months, and sometimes years.[6] Systemic manifestations are part of the syndrome in more than two thirds of the cases. Fever, fatigue, weight loss, vasculitis (including aortitis), pericarditis, arthralgia, lymphadenopathy, gastrointestinal and neurological symptoms may be present.[5,6]

Aortitis in Cogan's syndrome affects 10% of the individuals and has preference for the ascending aorta and aortic arch.[4-6] It is characterized by mixed inflammatory infiltrate without granuloma, with areas of necrosis and destruction of the wall,[4] and may cause dilation, aneurysm, aortic insufficiency and conduction blockages.[5] The patient in our report is 63 years old, older than the mean of patients with Cogan's syndrome. Nevertheless, this does not rule out our diagnosis and in fact makes the case even more unusual. The symptoms presented by the patient, bilateral hypoacusis progressing to deafness in the right ear, rotational vertigo, and hyperemia, pain and eye tearing, are compatible with the ocular and audiovestibular manifestations observed in the syndrome. Symptom progression, in our case, occurred more slowly. The pattern of aortitis involvement in our patient, from the root of the aorta to the middle portion of the aortic arch, is more classically similar to the pattern of aortitis in most cases of Cogan's syndrome.

It is thus clear that the diagnosis of Cogan's syndrome is clinical, and there is no established diagnostic test.[5] Due to the diagnostic difficulty, it is often recognized late, leading to a worse prognosis.[5-6] It can progress slowly and gradually to chronicity or recurrences.[5] Ocular changes are usually recurrent but present good resolution, usually causing no sequelae.[5,6] Hearing changes, on the other hand, have a worse prognosis and progress to deafness in 50% of the cases.[5,6] The presence of systemic manifestations such as vasculitis indicates a worse prognosis and a higher risk of complications, and may appear years after the initial symptoms.[5,6] Our patient progressed in a manner compatible with that described in the literature. He eventually had deafness in his right ear but no ocular sequelae. Although he did not develop severe cardiovascular symptoms, recurrent precordial pain for 10 years and intensified in the past 4 years was probably due to recurrent and profuse aortitis.

Treatment of aortitis caused by Cogan's syndrome is not well established. There is no clinical trial comparing therapeutic options.[5,6] Treatment consists basically of corticosteroids, and it may be necessary to associate with other immunosuppressants in more severe cases.[5,6] In cases of complicated aortitis, surgical procedure may be necessary.[5]

In the case reported, the patient presented significant clinical improvement with the introduction of prednisone 1 mg/kg/day, and no other immunosuppressants were required. He was discharged without symptoms and remained so until he returned for a medical visit two weeks later.

Conclusion

The case reports symptoms of precordial pain whose diagnosis was aortitis by Cogan's syndrome, a rare differential diagnosis. As mentioned above, Cogan's syndrome does not have a well-defined diagnosis or treatment, which can be recurrent and cause sequelae. The diagnosis of this patient was established years after the onset of the audiovestibular symptoms, when there were already auditory sequelae. Corticosteroid therapy yielded good results for the treatment of the vascular manifestation of the syndrome.

Resumo

Síndrome de Cogan – Uma aortite rara, de diagnóstico difícil, mas com potencial terapêutico

A inflamação da parede da aorta, denominada aortite, é uma condição clínica rara, que pode ser causada por diversas patologias, principalmente as de fundo inflamatório e/ou infeccioso. Nesse contexto, a ocorrência de sintomas vestibulares e oftalmológicos associados ao quadro remete ao diagnóstico de síndrome de Cogan, tornando o caso raro, mas passível de tratamento imunossupressor adequado e controle satisfatório da doença.

Palavras-chave: dor torácica, aortite, síndrome de Cogan.

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Pelvic floor muscle training for overactive bladder symptoms – A prospective study

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Summary

Introduction: Pelvic floor muscle training (PFMT) involves the contraction of the puborectal, anal sphincter and external urethral muscles, inhibiting the detrusor contraction, what justify its use in the treatment of overactive bladder (OAB) symptoms.
Objective: To verify the effects of isolated PFMT on the symptoms of OAB.
Method: Prospective clinical trial with 27 women with mixed urinary incontinence (MUI), with predominance of OAB symptoms and loss ≥ 2 g in the pad test. It was evaluated: pelvic floor muscles (PFMs) function (digital palpation and manometry); urinary symptoms (nocturia, frequency and urinary loss); degree of discomfort of OAB symptoms; and quality of life (Incontinence Quality-of-Life Questionnaire [I-QoL]). The PFMT program consisted of 24 outpatient sessions (2x/week + home PFMT). The Mann-Whitney and Wilcoxon tests (with a significance level of 5%) were used to analyse the data.
Results: There was a significant improvement of the urinary symptoms to the pad test (5.8±9.7, p<0.001), urinary loss (0.7±1.1, p=0.005) and nocturia (0.8±0.9, p=0.011). Reduction in the degree of discomfort of urinary symptoms was observed according to OAB-V8 questionnaire (10.0±7.7, p=0.001). There were also significant results in PFMs function: Oxford (3.6±0.9, p=0.001), endurance (5.2±1.8, p<0.001), fast (8.9±1.5, p<0.001) and manometry (26.6±15.8, p=0.003). In addition, quality of life had a significant improvement in the three domains evaluated by I-QoL.
Conclusion: The PFMT without any additional guidelines improves the symptomatology, the function of PFMs and the quality of life of women with OAB symptoms.

Keywords: urinary incontinence, pelvic floor, physical therapy modalities.

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Introduction

Overactive bladder (OAB) syndrome, also know as urgency syndrome or urgency-frequency syndrome, is a clinical diagnosis characterized by voiding urgency, with or without urgency urinary incontinence (UUI), usually accompanied by nocturia and increased voiding frequency, in the absence of infectious or metabolic factors.[1]

OAB symptoms compromise quality of life, causing social isolation, a decline in productivity, shame, frustration, anxiety and low self-esteem.[2] Symptoms include increased voiding frequency, present in approximately 85% of patients, followed by urgency, present in 54%.[3] UUI, in turn, is present in one third of the patients.[4] Enuresis, nocturia and urinary leakage due to stress incontinence are also reported.[5]

The main therapeutic modalities are conservative therapies, such as pharmacological treatment and behavioral therapy. Behavioral therapy includes the association of various resources, such as: educational program; changes in lifestyle; bladder training and urge suppression strategies; pelvic floor muscle training (PFMT); electro-stimulation; and programmed urination.[6-8]

The effects of behavioral therapy in the treatment of OAB symptoms are extensively studied and their efficacy, proven.[9] The literature investigating the effects of PFMT on OAB symptoms, without the association of other techniques, is scarce. The justification for using PFMT is that PFM contraction inhibits detrusor contraction, thus improving the symptoms of detrusor overactivity.[10] Accordingly, our main question is: Does PFMT alone result in improved symptoms of detrusor overactivity? Secondarily, we assessed the PFM function and the quality of life of women with OAB symptoms.

Method

Study design and population

This is a prospective clinical trial conducted from March 2011 to September 2013. The study was developed at the Urogynecology and Vaginal Surgery Unit of the General Gynecology Division, Department of Gynecology, at Universidade Federal de São Paulo (Unifesp). The Unifesp Research Ethics Committee granted approval for the study (protocol number CEP 1981/10). All participants signed the Voluntary Informed Consent Form, drafted in accordance with Resolution No. 196/96 of the National Health Council.

We included women with a history of mixed urinary incontinence (MUI) who reported OAB symptoms, with urinary leakage ≥ 2 g on the standardized volume pad test during medical appointment. Exclusion criteria were chronic-degenerative diseases; greater than grade II pelvic organ prolapse; urinary tract infection; neurological or psychiatric disease; present or past use of anticholinergics and tricyclic antidepressants during up to 6 months; or previous PFM training.

Once the participants were included, we initially collected their socio-demographic (age) and clinical (body mass index and urinary leakage time) information, as well as obstetric history (parity, vaginal delivery). Subsequently, the participating women were referred to a physiotherapist specialized in urogynecology, who assessed PFM function, urinary symptoms and quality of life.

PFM function assessment

PFM function was assessed by digital palpation, according to the PERFECT scheme. The Oxford scale was used for quantifying muscle strength. It assesses muscle strength from 0 to 5. Muscle endurance and rapid contractions were also assessed by means of the PERFECT system, an acronym meaning: Power (P), Endurance (E) and Fast (F), i.e. number of fast contractions.[11]

After digital examination, the maximum voluntary contraction (MVC) pressure of the pelvic floor muscles was assessed using a Peritron manometer (Cardio Design™, Victoria, Austrália). It is a conical vaginal catheter that is connected to a hand-held microprocessor with a latex tube, which allows pressure readings to be transmitted in centimeters of water (cmH_2O) when the catheter is compressed due to external pressure. The catheter was covered with a sterile condom and inserted into the vaginal canal until the entire length of the compressible part of the device was above the level of the hymenal annulus. The baseline pressure reading was recorded, and the catheter was then inflated to 100 cmH_2O and the device was then set to zero. Participants were requested to hold three consecutive MVCs, with a 10-second interval between each contraction[12] and the best of three was recorded.[13]

Assessment of urinary symptoms

OAB syndrome was assessed based on the Overactive Bladder Questionnaire (OAB-V8). This scale includes items on urgency, incontinence, nocturia and voiding frequency, as defined by the International Continence Society (ICS), referring to the previous four weeks. The final score is the sum of the partial scores obtained for each of the eight questions, ranging from 0 to 40. Patients with a final score of eight or more are considered as having OAB symptoms.[14]

Patients were also instructed to keep a voiding diary for seven days. In this diary, diurnal urinary frequency, nocturia and urinary leakage in situations of stress (coughing, sneezing, laughing, squatting, weight lifting, walking, and running) were recorded.[15]

We also conducted a standardized bladder volume pad test in order to analyze the severity of urinary incontinence. Firstly, the bladder was emptied with a catheter, and 250 mL of injection water were then infused. The participants used pre-weighted sanitary napkins and did the following physical activities: ten jumps, ten squats, ten coughs, climb and descend five flights of stairs ten times, walking for 15 minutes and washing hands for one minute. After the activities were completed, the pads were weighed again to determine the amount of leakage.[16]

Assessment of quality of life

The impact of OAB syndrome symptoms on quality of life was assessed based on the Incontinence Quality-of-Life (I-QoL) Questionnaire. The I-QoL Questionnaire consists of 22 questions that assess the limitations on human behavior, the psychosocial impact and social constraint brought about by urinary incontinence. Responses are given on a scale ranging from 1 to 5 points and the final scores are then summed up and converted into percentages. The higher the percentage, the better the quality of life.[17]

PFM training protocol

The treatment consisted of outpatient sessions conducted under the supervision of the physiotherapist (24 outpatient sessions of 40 minutes each) and home training for 12 weeks.

A program consisting of three sets of ten repetitions per day was prescribed. The treatment protocol was personalized and based on the initial evaluation as assessed by PERFECT. We instructed our patients to contract and hold the contraction for some seconds (the time corresponds to the reading obtained during the initial assessment – from 1 to 10 seconds), which were then followed by fast contractions (calculated in the same way). One series of exercises consisted of ten repetitions of each movement (endurance + rest + fast contractions). For example, if 6 seconds of muscle endurance and three fast muscle contractions were observed in the PERFECT system, we instructed our patients to hold maximum voluntary contraction for 6 seconds, and twice as long the second time (12 seconds), finally followed by three rapid contractions (Figure 1). PFM function assessment by the PERFECT system took place monthly for training adjustments and progression.[18] No additional resources were addressed for treating OAB syndrome symptoms in addition to PFMT.

We instructed our patients to use the same protocol they learned during the outpatient sessions daily but at home, in the supine (first month), seated (second month) and orthostatic (third month) positions (Figure 2).

Adherence to the PFM training program

To monitor adherence to home exercises, patients completed an exercise diary. Adherence to the training protocol was based on the daily PFM training records performed by the patients (three sets of daily exercises). We instructed patients to record exercises only when they actually practiced them. Adherence was expressed as the average of series of exercises patients did monthly during the treatment period.[19] Outpatient attendance at sessions was monitored by the physiotherapist accompanying the patients during treatment and expressed as an average at the end of the three-month treatment.

For the statistical analysis, we used the SPSS software version 22.0. For analyzing demographic data, clinical traits and adherence to the exercises, we used the Mann-Whitney test. In order to analyze possible differences before and after treatment, we used the Wilcoxon test with a significance level of 5%.

Results

Twenty-seven (27) patients were included in the period between March 2011 and September 2013. Seven women did not complete the treatment (35% dropout rate). Reasons reported for dropout were: two patients reported difficulties in obtaining release from work; three due to family reasons; and another two due to prioritizing the treatment of other health conditions. Demographic and clinical traits are described in Table 1.

In comparing the urinary symptoms between the initial and final assessments conducted during the treatment, we observed significant improvement in the severity of UI as assessed in the pad test; in urinary leakage and nocturia, as evaluated with the voiding diary; in OAB symptoms as assessed by the OAB-V8 questionnaire; and in the three domains of quality of life assessment as evaluated by the I-QoL questionnaire (Table 2).

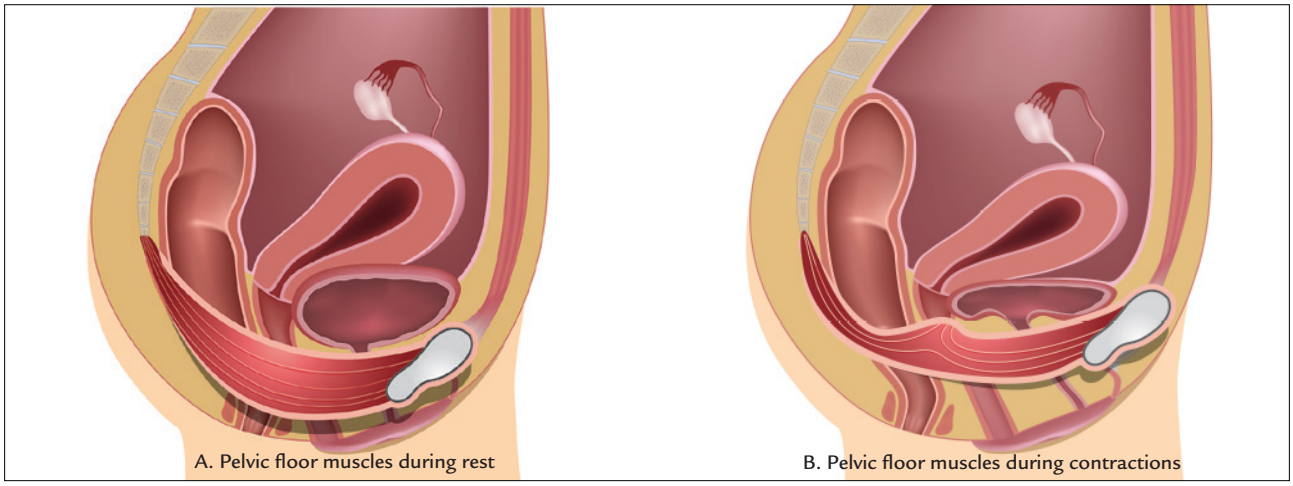

FIGURE 1 Illustration of pelvic floor muscles at rest (A) and during contraction (B).

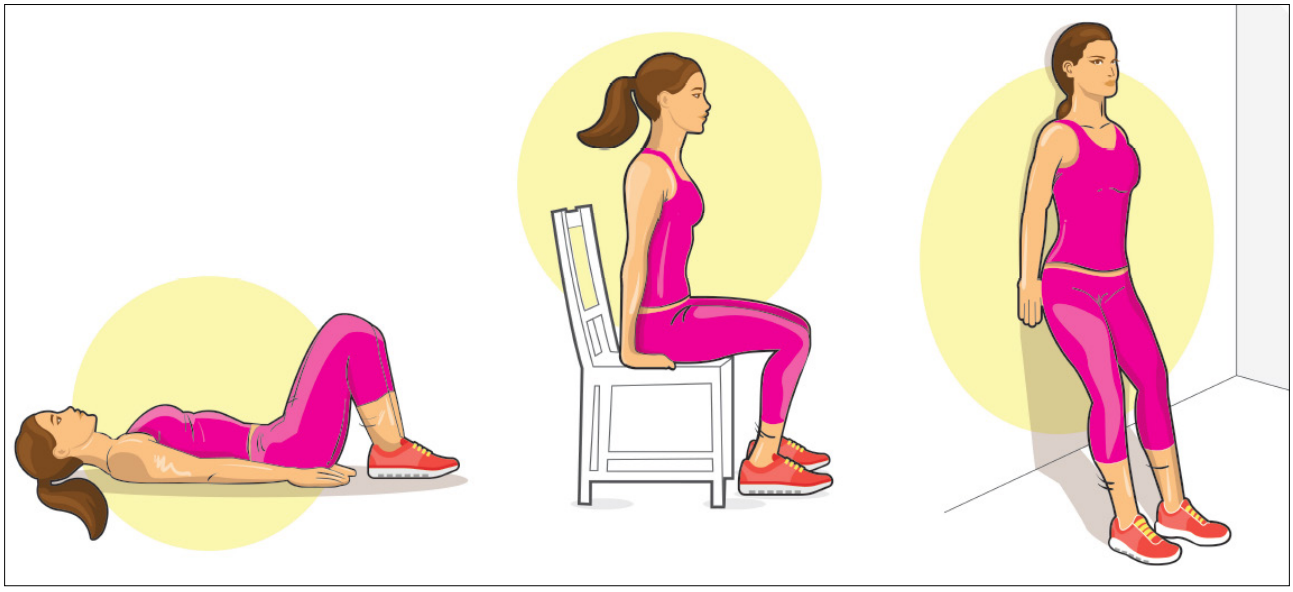

FIGURE 2 Illustration of patient positioning for performing exercises at home.

TABLE 1 Clinical and demographic characteristics of the patients.

Variables (n=20)	Mean (minimum–maximum)
Age (years)	59.9 (41-77)
BMI (kg/m^2)	27.4 (21-38)
Duration of symptoms (months)	128.4 (12-420)
Pregnancies (n)	5.1 (2-16)
Parity (n)	3.5 (0-12)

Mann-Whitney; n: sample size; BMI: body mass index.

TABLE 2 Assessment of urinary symptoms (pad test, voiding diary, OAB-V8) and quality of life before and after treatment.

Variables (n=20)	Baseline (mean±SD)	Final (mean±SD)	p
Pad test (g)	19.5±22.2	5.8±9.7	<0.001*
Urinary leakage	2.1±1.6	0.7±1.1	0.005*
Frequency	7.4±2.8	6.7±2.2	0.360
Nocturia	1.6±1.6	0.8±0.9	0.011*
OAB-V8	21.9±7.2	10.0±7.7	<0.001*
Limitations on human behavior	103.2±28.8	135.5±21.3	0.001*
Psychosocial impact	140.5±36.8	170.5±29.2	0.005*
Embarrasment and social constraint	44.5±30.7	59.7±28.3	0.024*

Wilcoxon*; SD: standard deviation; n: sample size; g: grams; OAB-V8: Overactive Bladder Questionnaire; I-QoL: Incontinence Quality-of-Life Questionnaire.

During PFM assessment, we observed improvement in muscle function when comparing the initial and final assessments conducted during the treatment, evaluated with the PERFECT system and the manometer (Table 3).

The mean outpatient attendance during outpatient PFMT was 18.4±4.6 sessions. Adherence to in-home exercises (series of exercises completed) was: 78.3±15.5 (30-82) in the first month; 74.6±16.3 (24-82) in the second month; and 76.2±16.0 (24-82) in the third month.

Discussion

PFMT is among the types of conservative treatment for urinary symptoms. This is a low-cost, first-line treatment

TABLE 3 Assessment of the function of pelvic floor muscles (manometry and PERFECT) before and after treatment.

Variables (n=20)	Baseline (mean±SD)	Final (mean±SD)	p
MVC (cmH_2O)	19.7±12.2	26.6±15.8	0.003*
Oxford	2.6±1.1	3.6±0.9	0.001*
Endurance (s)	2.7±1.2	5.2±1.8	<0.001*
Fast	5.3±2.4	8.9±1.5	<0.001*

Wilcoxon; SD: standard deviation; s: seconds; n: sample size; MVC: maximum voluntary contraction.

for women with stress urinary incontinence (SUI).[8] Its success rates range from 60 to 75%.[15,20]

In addressing OAB symptoms, behavioral therapy programs appear as first-rate treatments. These programs include the association of techniques that aim at minimizing or even eliminating the symptoms displayed by the patient. Behavioral therapy programs include: educational program, changes in lifestyle, bladder training and urge suppression strategies, encouragement and positive reinforcement, programmed urination, PFMT, PFMT with biofeedback (BF) and PFMT with electrostimulation.[6] In our study, we proposed to use only PFMT for the treatment of OAB symptoms. We observed a significant improvement in PFM function, urinary symptoms and quality of life after 12 weeks of outpatient and in-home training.

Studies published in the literature that make use of PFMT alone for OAB symptoms are scarce. In our study, we observed an improvement in muscle function as measured by digital palpation (p<0.001) and manometry (p<0.001). Wang et al. used the isolated PFMT, training with BF, and coupled with electrostimulation without combining other resources to suppress urgency and urge incontinence. Improvement in PFM function was found across all three groups, with greater relevance in the BF group. The increase in vaginal contraction pressure was 105% in the BF group; 78.9% in the PFMT group; and only 12.6% in the electrostimulation group.[21] BF is an adjuvant to PFMT and allows patients to observe PFM contractions during exercise, thereby improving exercise performance and treatment motivation,[22-24] which may justify a significant improvement in PFM function.

However, Wang et al.[21] do not consider muscle strength the best indicator when assessing OAB symptoms. Treatment of overactive bladder symptoms should be evaluated by its effectiveness in reducing urinary symptoms and its impact on quality of life.

In our study, we observed an improvement in urinary leakage as assessed by pad test (p<0.001), voiding diary (p=0.005), nocturia (p=0.011) and degree of discomfort caused by urinary symptoms as assessed by the OAB-V8 questionnaire (p<0.001). In assessing quality of life, we also observed positive results in the three domains evaluated by the I-QoL questionnaire. There was no significant reduction in urinary frequency, given that initial values were within normal standards, according to the International Urogynecological Association (IUGA)/International Continence Society (ICS).[1]

Wang et al.[21] found urinary symptom reduction rates of about 51% in the electrostimulation group, 50% in the isolated PFMT group and 38% in the PFMT group with BF. The authors found electrostimulation to be superior to the other groups. Electrostimulation inhibits detrusor activity by directly stimulating the pudendal nerve.[25] Burgio et al.[26] observed incontinence reduction of about 80% in women with urge incontinence by using PFMT with BF.

In a study with the specific objective of evaluating and comparing the effects of oxybutynin, electrostimulation and perineal exercises in the treatment of detrusor overactivity, the urge incontinence decreased in all three groups (p<0.05) following treatment as assessed by the voiding diary. Urgency disappeared in about 63% of patients in the oxybutynin group; in 52% of patients in the electrostimulation group; and in 57% of patients in the perineal exercises group. The results were similar between the groups (p=0.754). In the subjective evaluation, the percentage of women who were satisfied after treatment was 77%, 52% and 76%, respectively, in the oxybutynin, electrostimulation and perineal exercise groups, with no significant differences between them (p=0.142).[5]

The benefits of short- and long-term physiotherapeutic treatment programs depend on patient adherence.[27] In our study, we observed an outpatient attendance of 75% and a mean in-home adherence during the 3 month treatment of 80%.

The results from these studies should be compared with caution, as there are differences in the inclusion criteria and disease definitions across them, as well as specific criteria for evaluating cure and improvement in symptoms. Most studies combined treatments, which makes it difficult to understand how each of the resources can act individually. In our sample, the patients did not receive any information on behavioral orientation.

There is insufficient evidence to support PFMT in treating OAB symptoms, since it is unclear how PFM contraction can inhibit detrusor contractions. PFMT involves the contraction of the puborectalis muscles, as well as the anal and external urethral sphincters. Studies have shown that contraction of these muscles leads to suppression of detrusor contraction.[28,29] The studies published in the literature that use PFMT for OAB are scarce, and most of them rely on the association of different treatment modalities.

Our study is a prospective trial that shows benefits of isolated PFMT in improving urinary leakage symptoms, nocturia, quality of life and PFM function. Nonetheless, randomized controlled trials are needed to further demonstrate and understand how women with OAB symptoms can benefit from PFMT.

Conclusion

Our results suggest that PFMT with no additional guidelines can improve urinary leakage, nocturia, PFM function and quality of life in women with OAB symptoms. Still, randomized controlled trials using PFMT alone are required in order to better demonstrate and understand how it can act on OAB symptoms.

Resumo

Treinamento dos músculos do assoalho pélvico nos sintomas da bexiga hiperativa – Um estudo prospectivo

Introdução: O treinamento dos músculos do assoalho pélvico (TMAP) envolve a contração dos músculos puborretal, esfíncteres anal e uretral externo, inibindo a contração do detrusor, o que justifica sua utilização no tratamento dos sintomas da bexiga hiperativa (BH).
Objetivo: Verificar os efeitos do TMAP isolado sobre a sintomatologia da BH.
Método: Ensaio clínico prospectivo com 27 mulheres com incontinência urinária mista (IUM), com predomínio de sintomas de BH e perda ≥ 2 g no *pad test*. Avaliaram-se: função dos músculos do assoalho pélvico (MAP) (palpação digital e manometria); sintomas urinários (noctúria, frequência e perda urinária); grau de incômodo dos sintomas de BH (Overactive Bladder Questionnaire [OAB-V8]); e qualidade de vida (Incontinence Quality-of-Life Questionnaire [I-QoL]). O programa de TMAP consistiu em 24 sessões ambulatoriais (2x/semana + TMAP domiciliar). Os testes de Mann-Whitney e Wilcoxon (com nível de significância de 5%) foram utilizados para analisar os dados.
Resultados: Observou-se melhora significativa dos sintomas urinários ao *pad test* (5,8±9,7; p<0,001); ao diário miccional (perda urinária [0,7±1,1; p=0,005] e noctúria [0,8±0,9; p=0,011]). Foram observados redução do grau de incômodo dos sintomas urinários conforme questionário OAB-V8 (10,0±7,7; p=0,001) e significativos resultados na função dos MAP: Oxford (3,6±0,9; p=0,001), Endurance (5,2±1,8; p<0,001), Fast (8,9±1,5; p<0,001) e manometria (26,6±15,8; p=0,003). No mais, a qualidade de vida teve significativa melhora nos três domínios avaliados pelo I-QoL.
Conclusão: O TMAP sem quaisquer orientações adicionais melhora a sintomatologia, a função dos MAP e a qualidade de vida de mulheres com sintomas de BH.

Palavras-chave: incontinência urinária, diafragma da pelve, modalidades de fisioterapia.

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Practices and obstetric interventions in women from a state in the Northeast of Brazil

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Summary

Objective: To describe practices and interventions used during labor and childbirth and factors associated with such practices in puerperae in the state of Sergipe.
Method: A cross-sectional study with 768 postpartum women from 11 maternity hospitals interviewed 6 hours after delivery, and hospital records review. The associations between best practices and interventions used during labor and delivery with exposure variables were described using simple frequencies, percentages, crude and adjusted odds ratio (ORa) with the confidence interval.
Results: Of the women in the study, 10.6% received food and 27.8% moved during labor; non-pharmacological methods for pain relief were performed in 26.1%; a partogram was filled in 39.4% of the charts; and an accompanying person was present in 40.6% of deliveries. Oxytocin, amniotomy and labor analgesia were used in 59.1%, 49.3% and 4.2% of women, respectively. Lithotomy position during childbirth was used in 95.2% of the cases, episiotomy in 43.9% and Kristeller maneuver in 31.7%. The variables most associated with cesarean section were private financing (ORa=4.27, 95CI 2.44-7.47), higher levels of education (ORa=4.54, 95CI 2.56-8.3) and high obstetric risk (ORa=1.9, 95CI 1.31-2.74). Women whose delivery was funded privately were more likely to have an accompanying person present (ORa=2.12, 95CI 1.18-3.79) and to undergo labor analgesia (ORa=4.96, 95CI 1.7-14.5).
Conclusion: Best practices are poorly performed and unnecessary interventions are frequent. The factors most associated with c-section were private funding, greater length of education and high obstetric risk.

Keywords: maternal and child health, labor, obstetric, delivery, parturition, episiotomy, cesarean section.

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Introduction

The model for parturition care in Brazil until the mid-twentieth century was one that relied mostly on home care, which was provided mainly by female accoucheuses and midwives. Since then, there has been a process of hospitalization and medicalization of delivery with a large increase in the rates of interventions such as episiotomy, use of oxytocin and cesarean section.[1,2] The trend towards an increase in the number of cesarean sections is observed worldwide – except for the Netherlands and other European countries, where it is minor – with Brazil as a leader.[3]

Two decades ago, the World Health Organization (WHO) published the report "Safe Motherhood" with recommendations on appropriate obstetric practices and those to be avoided.[4] Evidence based on systematic reviews suggests that ambulating and staying in upright positions during the first period of labor reduces the duration of labor, the risk of cesarean section and the use of analgesia, while not posing risks to the mother or the newborn.[5] There is no justification for restricting liquids or foods during labor for women at low risk of complications.[6] Likewise, there is no evidence to support routine amni-

otomy, since this does not reduce the duration of labor or the cesarean section rate.[7] The presence of a support person (doula) during labor is associated with reduction in the use of analgesia, lower rate of operative delivery and greater maternal satisfaction.[8] If their presence is not possible, women should be encouraged to choose an accompanying person (relative or friend).[9] Performing selective episiotomy is associated with less posterior perineal trauma, less need for suturing and fewer complications, with no difference in pain intensity and severe vaginal or perineal trauma when compared to routine episiotomy.[10] The Kristeller maneuver, albeit safe for the fetus, increases the risk of vaginal and cervical laceration and should thus be avoided.[11]

In order to improve prenatal care and delivery and adapt obstetric practices to current recommendations, the Brazilian Ministry of Health has been adopting several public policies: the National Prenatal and Birth Program (PHPN) by means of the Ordinance GM No. 569, of June 1, 2000;[12] the guarantee of right to an accompanying person during labor, childbirth and immediate puerperium (Law 11,108, of April 7, 2005)[13] and the so-called Stork Network (Rede Cegonha, Ordinance No. 1459, of June 24, 2011), which reinforces and expands on the PHPN proposal,[14] as well as the definition of the National Directive for Normal Labor Care issued by the National Technology Incorporation Commission (CONITEC) of the Unified Health System (SUS, in the Portuguese acronym) (CONITEC- SUS, 2016).[2]

Data from the "Birth in Brazil" (Nascer no Brasil) study showed that the prevalence of good practices in conducting labor was lower in the North and Northeast regions.[15] The state of Sergipe is the smallest in Brazil, located in the Northeast region. In 2014, 34,369 live births and a maternal mortality rate of 61/100,000 live births (DATASUS, the SUS Department of Informatics)[16] were recorded, well above that which is considered acceptable by the WHO, namely up to 20/100,000. There is no study with a representative sample at the state level evaluating the use of "good practices" and interventions in childbirth. The purpose of this study was to describe adequate practices used during labor/delivery (feeding, movement, use of non-pharmacological methods for pain relief, monitoring of labor using partogram and the presence of an accompanying person during labor) and interventions aimed at women (use of oxytocin, amniotomy, analgesia, episiotomy, Kristeller maneuver, lithotomy delivery, and cesarean section). It also aimed at investigating factors associated with such practices in puerperae at 11 maternity hospitals in the state of Sergipe.

Method

This is a cross-sectional study extracted from the "Birth in Sergipe" (Nascer em Sergipe), a cohort study conducted between June 2015 and April 2016. The local team was trained by investigators from Fundação Osvaldo Cruz (Fiocruz), who participated in the national study, to evaluate a sample representative of the state of Sergipe. The method used in the "Birth in Brazil"[17] study was reproduced. Public, private and mixed maternities that had more than 500 births per year were included.

Sample size was calculated considering a cesarean section rate of 38% (DATASUS, 2011),[16] totaling 358 women to be interviewed. This figure was calculated as an estimate of cesarean section prevalence considering all institutions with 500 or more births in the state of Sergipe, with a 95% confidence interval. To increase the power of the study and evaluate associations with other variables, we doubled the sample to 768 puerperae and their conceptuses. An allocation proportional to the size of the institution was adopted for distributing the calculated sample size.

The interviewers stayed at least 7 days at each institution. If the number of puerperae was reached before the end of that period, a random number limiting the maximum daily number of interviewees would be drawn up so as to ensure that all 7 scheduled days were reached. We included all randomly selected women who had been admitted to chosen maternities at the time of delivery and their conceptuses, alive or dead, with birth weight greater than or equal to 500 g and/or gestational age greater than or equal to 22 weeks, provided they accepted to participate in the study and sign a Voluntary and Informed Consent Form (VICF). We excluded the women who could not speak and/or understand Portuguese or who could not be reached by telephone between 45-60 days and 6 to 8 months postpartum. No puerpera that was considered eligible refused to participate. There were two eligible patients who were lost due to complications in the immediate puerperium and progressed to death.

Face-to-face interviews with the puerperae were carried out between 6 and 24 hours after delivery. Data was extracted from woman's and newborn's records after discharge (or death). In the case of prolonged hospital stay, data was extracted 42 days after admission to hospital and 28 days after the newborn's birth. Their prenatal cards were photographed, and the information were entered into the database. Follow-up phone interviews were conducted at 45-60 days and 6 to 8 months after delivery to investigate maternal and neonatal complications in the short and medium terms.

The (independent) exposure variables studied were the source of financing: public (SUS system) or private (private or granted by health insurance companies); locality: capital or countryside; age (10 to 19; 20 to 34; > 35); number of years of formal education (7 or less; 8-10; 11 or more); self-reported skin color (white or non-white); number of previous deliveries (0; 1-2; 3 or more); and usual or high obstetric risk (gestational diabetes, gestational or pre-gestational high blood pressure, obesity, AIDS, gestational age lesser than 37 weeks or greater than 41 weeks at the time of birth, multiple pregnancy, non-cephalic presentations, birth weight less than 2,500 g or greater than 4,499 g and below the fifth-percentile or above the ninety-fifth percentile of weight for gestational age). It was used the same risk classification as the one adopted in the "Birth in Brazil" study.[18]

The outcomes evaluated (dependent variables) were "good practices" and obstetric interventions during labor/delivery. The good practices during labor/delivery investigated were: feeding, movement during labor, use of non-pharmacological procedures for pain relief, use of partogram and presence of an accompanying person, whereas interventions during labor/delivery were: use of oxytocin, amniotomy, epidural analgesia, elective or intrapartum cesarean section (i.e. in patients who went into labor), episiotomy, use of the Kristeller maneuver, and delivery in the lithotomy position.

For statistical analysis, the associations between good practices and interventions used during labor and delivery with exposure variables were described by simple frequencies, percentages, crude odds ratios, as well as their confidence interval. In the multivariate analysis, odds ratios were adjusted by using a generalized linear model with Bernoulli distribution (logistic regression) with robust standard errors. Occasionally, odds ratios could not be calculated due to the presence of null crossings between variables (separation problems).[19]

The project was approved by the Ethics Committee for Research involving human beings at the Universidade Federal de Sergipe, CAAE: 22488213.4.0000.5546. The participants' identity and rights have been preserved. This research project follows the recommendations of Normative 196/96 pursuant to Resolution No. 466/2012, which has replaced Resolution No. 196, of October 10, 1996, of the National Health Council of the Ministry of Health (Conselho Nacional de Saúde do Ministério de Saúde), Brasília, DF, Brazil. The puerperae signed a VICF, having a guaranteed right to terminate participation at any moment, without suffering any damage.

Results

The participants' mean age was 25 years: 21.4% were adolescents and 7.3% were aged 35 years or more. Brown skin was the most frequently self-reported skin color (75.1%), with yellow and indigenous skin colors corresponding to 3% and 0.4%, respectively. With regard to education, 48.7% had 7 or less years of formal education and 33.2% had 8-10 years. Most participants performed no paid work (55.5%) and lived with their partner (84.3%). Regarding parity, 43.2% were primiparous and 17.1% had three or more previous pregnancies. Delivery was normal in 59.4% and cesarean section was performed in 40.46% of cases. According to the type of financing involved, the cesarean section rate was 35% at public health care services and 75% at private ones.

Feeding and movement during labor, use of non-pharmacological measures for pain relief, use of partogram for labor monitoring, use of oxytocin, epidural analgesia and intrapartum cesarean section were evaluated in the 566 (73.6%) patients who went into labor. As far as interventions at delivery, episiotomy, Kristeller maneuver and delivery in the lithotomy position were evaluated in the 456 (59.4%) patients who had normal delivery. The presence of an accompanying person during delivery and elective cesarean section were evaluated among all patients in the study. Amniotomy was evaluated in 389 patients who went into labor, and those who had premature rupture of membranes before admission were excluded.

In 10.6% of cases, women were given food and 27.8% moved during labor; non-pharmacological measures for pain relief were taken in 26.1%; the partogram was filled out in 39.4% of medical records; and the accompanying person was present in 40.6% of deliveries. With respect to interventions, oxytocin was used in 59.1% and amniotomy in 49.3% of cases. Labor analgesia was provided to 4.2% of women. Delivery occurred in the traditional lithotomy position in 95.2% of cases; episiotomy was performed in 43.9% and Kristeller maneuver, in 31.7%. Among the patients who went into labor, 14.3% required resolution by cesarean section, whereas 26.3% of women participating in the study had elective cesarean section. Most deliveries (75%) were performed by a medical obstetrician and 17% by a nurse obstetrician.

The associations between independent (sociodemographic) variables and the outcomes assessed: "good practices" and interventions during labor and delivery can be observed, respectively, in Tables 1, 2 and 3. After adjusting for confounding variables, we found that having a privately funded birth was associated to greater likelihood of having an accompanying person during labor (ORa=2.12;

95CI 1.18-3.79) (Table 1), use of analgesia (ORa=4.96; 95CI 1.7-14.5) (Table 2), indication of intrapartum cesarean section (ORa=5.89; 95CI 3.11-11.1); or elective cesarean section (ORa=4.27; 95CI 2.44-7.47) (Table 3) and less chance of using oxytocin (ORa=0.23; 95CI 0.1-0.52) (Table 2). Patients in the countryside are more likely to eat (ORa=2.85; 95CI 1.5-5) and to have their labor monitored by a partogram (ORa=4.34; 95CI 3-6.6) (Table 1), but were also the ones who underwent Kristeller maneuver more often (ORa=1.7; 95CI 1.1-2.6) (Table 3), whereas those in the capital were the most likely to have the presence of an accompanying person during labor (ORa=5.76; 95CI 3.86-8.58) (Table 1), be given oxytocin (ORa=4.17; 95CI 2.8-6.21) and labor analgesia (ORa=7.38; 95CI 1.65-33.1) (Table 2). Regarding age, women between 10 and 34 years of age underwent a greater number of non-pharmacological procedures for pain relief (ORa=3.12; 95CI 1.14-8.51) (Table 1) whereas in those aged between 10 and 19 years a larger number of lithotomy deliveries (ORa=16.28; 95CI 1.75-151) was performed compared with those aged 35 years or older (Table 3). As for schooling, those who had 11 or more years of formal education reported the presence of an accompanying person during delivery more frequently than those who had up to seven years of education (ORa=3.12; 95CI 1.6-5.88) (Table 1), as well as more elective c-sections (ORa=4.54; 95CI 2.56-16.6) (Table 3). Women with one or two previous deliveries reported more frequently having an accompanying person during labor than those with three or more previous deliveries (ORa=1.88; 95CI 1.2-2.7) (Table 1). They also underwent Kristeller maneuvers less often than those with three or more previous deliveries (ORa=0.52; 95CI 0.34-0.81), whereas these, in turn, were at a higher risk of episiotomy than those with one to two previous deliveries (ORa=1.7; 95CI 1.16-2.77) (Table 3). Finally, pregnant women classified as being at a high risk stood a higher chance of eating during labor (ORa=2.38; 95CI 1.36-4.16); recorded more deliveries monitored by a partogram (ORa=2.22; 95CI 1.46-3.39) (Table 1) and had a higher number of intrapartum (ORa=2.24; 95CI 1.44-3.47) and elective (ORa=1.9; 95CI: 1.31-2.74) c-sections than did pregnant women at low-risk (Table 3).

Discussion

Periodic reviews of practices used in obstetric care should be conducted to verify whether the recommendations of reviews and international[9] and national[2] guidelines are being followed.

The so-called "good practices" recommended for conducting labor were used in less than 30% of women in the state of Sergipe, whereas interventions that should not be used due to lacking evidence that supported the need thereof and/or due to proven damage such as the use of oxytocin, amniotomy, episiotomy, Kristeller maneuver (which is strongly discouraged) and lithotomy delivery[2] were very frequently performed. This result is similar to that found by the "Birth in Brazil" study, which detected that good practices are used in less than 50% of deliveries and interventions such as Kristeller maneuver (37%) and episiotomy (56%) are still widely practiced.[15]

In the state of Sergipe, as much as in Brazil, there is great diversity in the care of patients in the public health care system when compared to the private system. Women receiving care in the private health care system had more comfortable deliveries, with a more frequent use of analgesia and a less frequent use of oxytocin. In addition, they had the presence of accompanying persons more frequently, unlike those receiving care in the public health care system, who received oxytocin more often as well as less analgesia, characterizing the practice of medicalized and painful deliveries. However, the indications of cesarean section, either electively or during labor, were more frequent in women receiving care at the private health care system, from the capital city of Sergipe, with greater lengths of formal education and at a high obstetric risk. A Spanish study has shown that patients from the private health care system and private hospitals with fewer births are more likely to undergo cesarean section and invasive procedures such as episiotomy or instrumental vaginal delivery.[20] Another study evaluated the role played by the source of payment in increasing cesarean section rates in Brazil between 1998 and 2008 and showed that private payment was associated with higher cesarean rates, as well as an older age, greater length of formal education and residing outside the Northeast region.[21]

A national survey carried out in Brazil in 2010 also showed a higher frequency of cesarean delivery among older women and those with greater lengths of formal education, primiparous, whose prenatal care was provided by the private health care system and residing in the South, Southeast and Midwest[22] regions. A study published in 2014 shows a change in this geographical pattern, with the frequency of cesarean sections being higher in the North and lower in the Southeast regions, despite the fact that the latter has the highest coverage by health insurance companies in Brazil. This may indicate the beginning of a change due to the movements in favor of humanized delivery and greater access to evidence-based information on good obstetric practices.[15]

We can attribute the reasons for a higher frequency of cesarean sections among women receiving care in the pri-

TABLE 1 Crude (OR) and adjusted odds ratios (ORa) for sociodemographic determinants of good practices during labor and delivery in women in the state of Sergipe, 2015.

	Eating allowed OR (95CI)	Non-pharmacological procedures Pain relief OR (95CI)	Moving freely OR (95CI)	Partogram OR (95CI)	Accompanying person during labor OR (95CI)
Funding source					
Private	0.19 (0.03-1.43)	0.54 (0.24-1.25)	0.59 (0.27-1.30)	0.61 (0.26-1.41)	6.66 (4.09-10.8)
Public	1	1	1	1	1
Location					
State capital	0.42 (0.24-0.74)	0.96 (0.66-1.40)	0.75 (0.51-1.09)	0.26 (0.18-0.38)	7.01 (4.87-10.1)
Countryside	1	1	1	1	1
Age					
10 to 19	1.03 (0.35-3.00)	3.12 (1.14-8.51)	1.03 (0.49-2.17)	0.76 (0.36-1.62)	0.83 (0.46-1.52)
20 to 34	0.90 (0.34-2.43)	3.00 (1.15-7.81)	0.91 (0.46-1.81)	0.87 (0.43-1.75)	0.94 (0.55-1.62)
> 35	1	1	1	1	1
Years of education					
0 to 7 years	1.48 (0.43-5.07)	1.19 (0.56-2.55)	1.14 (0.55-2.40)	0.95 (0.46-1.96)	0.17 (0.10-0.29)
8 to 10 years	1.34 (0.38-4.71)	0.84 (0.38-1.83)	0.78 (0.36-1.68)	0.75 (0.36-1.59)	0.28 (0.17-0.46)
> 11 years	1	1	1	1	1
Skin color					
White	1.75 (0.88-3.48)	1.18 (0.69-2.02)	1.24 (0.73-2.09)	1.34 (0.79-2.27)	1.07 (0.66-1.57)
Non-white	1	1	1	1	1
Parity					
0	0.71 (0.16-3.16)	0.91 (0.34-2.40)	0.63 (0.22-1.75)	1.22 (0.48-3.14)	0.86 (0.42-1.80)
1 to 2	0.63 (0.35-1.14)	0.67 (0.45-1.01)	0.62 (0.42-0.93)	1.04 (0.71-1.52)	0.70 (0.51-0.96)
> 3	1	1	1	1	1
Obstetric risk					
Usual (low risk)	1	1	1	1	1
Unusual (high risk)	1.89 (1.09-3.29)	1.02 (0.68-1.54)	1.01 (0.67-1.51)	1.65 (1.11-2.44)	0.84 (0.60-1.16)
	ORa (95CI)	**ORa (95CI)**		**ORa (95CI)**	**ORa (95CI)**
Funding source					
Private					2.12 (1.18-3.79)
Public					1
Location					
State capital	0.35 (0.20-0.63)			0.23 (0.15-0.33)	5.76 (3.86-8.58)
Countryside	1			1	1
Age					
10 to 19		3.12 (1.14-8.51)			
20 to 34		3.00 (1.15-7.81)			
> 35		1			
Years of education					
0 to 7 years					0.32 (0.17-0.61)
8 to 10 years					0.42 (0.23-0.77)
> 11 years					1
Parity					
0					0.66 (0.29-1.50)
1 to 2					0.53 (0.36-0.78)
> 3					1
Obstetric risk					
Usual (low risk)	1			1	
Unusual (high risk)	2.38 (1.36-4.16)			2.22 (1.46-3.39)	

OR: crude odds ratio; ORa: adjusted odds ratio; 95CI: 95% confidence interval.

TABLE 2 Crude (OR) and adjusted odds ratios (ORa) for sociodemographic determinants of interventions during labor and delivery in women in the state of Sergipe, 2015.

	Oxytocin OR (95CI)	Analgesia OR (95CI)	Amniotomy OR (95CI)
Funding source			
Private	0.53 (0.24-1.18)	14.3 (4.69-43.8)	1.45 (0.49-4.28)
Public	1	1	1
Location			
State capital	3.79 (2.55-5.62)	14.9 (1.95-113)	0.86 (0.55-1.34)
Countryside	1	1	1
Age			
10 to 19	1.48 (0.68-3.22)	*	1.35 (0.52-3.48)
20 to 34	1.16 (0.57-2.37)	*	1.39 (0.57-3.39)
> 35	1	1	1
Years of education			
0 to 7 years	1.99 (0.93-4.29)	0.08 (0.02-0.36)	1.20 (0.46-3.13)
8 to 10 years	2.36 (1.08-5.19)	0.35 (0.10-1.12)	1.45 (0.54-3.85)
> 11 years	1	1	1
Skin color			
White	0.70 (0.41-1.18)	1.52 (0.42-5.45)	1.20 (0.64-2.27)
Non-white	1	1	1
Parity			
0	0.66 (0.25-1.76)	2.56 (0.30-22.2)	1.14 (0.37-3.52)
1 to 2	0.80 (0.54-1.18)	1.87 (0.67-5.26)	0.77 (0.49-1.22)
> 3	1	1	1
Obstetric risk			
Usual (low risk)	1	1	1
Unusual (high risk)	1.04 (0.69-1.57)	1.45 (0.52-4.08)	0.77 (0.47-1.24)
	ORa (95CI)	**ORa (95CI)**	
Funding source			
Private	0.23 (0.10-0.52)	4.96 (1.70-14.5)	
Public	1	1	
Location			
State capital	4.17 (2.80-6.21)	7.38 (1.65-33.1)	
Countryside	1	1	

OR: crude odds ratio; ORa: adjusted odds ratio; 95CI: 95% confidence interval.
*OR could not be calculated due to numerical problems.[19]

TABLE 3 Crude (OR) and adjusted odds ratios (ORa) for sociodemographic determinants of interventions during delivery in women in the state of Sergipe, 2015.

	Kristeller OR (95CI)	Lithotomy OR (95CI)	Episiotomy OR (95CI)	C Intrapartum OR (95CI)	C Elective OR (95CI)
Funding source					
Private	1.61 (0.63-4.09)	0.91 (0.12-7.19)	1.80 (0.71-4.59)	5.48 (2.90-10.3)	5.50 (3.59-8.43)
Public	1	1	1	1	1
Location					
State capital	0.57 (0.38-0.85)	1.00 (0.43-2.37)	0.73 (0.48-1.10)	2.11 (1.35-3.27)	1.20 (0.87-1.66)
Countryside	1	1	1	1	1

(*continues*)

TABLE 3 Crude (OR) and adjusted odds ratios (ORa) for sociodemographic determinants of interventions during delivery in women in the state of Sergipe, 2015.

	Kristeller OR (95CI)	Lithotomy OR (95CI)	Episiotomy OR (95CI)	C Intrapartum OR (95CI)	C Elective OR (95CI)
Age					
10 to 19	1.50 (0.66-3.44)	16.28 (1.75-151)	2.98 (1.15-7.68)	0.40 (0.18-0.89)	0.37 (0.20-0.70)
20 to 34	0.92 (0.42-2.01)	2.30 (0.73-7.31)	2.17 (0.89-5.32)	0.68 (0.34-1.35)	0.74 (0.44-1.24)
> 35	1	1	1	1	1
Years of education					
0 to 7 years	0.56 (0.25-1.28)	2.88 (0.75-11.1)	0.76 (0.32-1.85)	0.32 (0.16-0.64)	0.13 (0.08-0.22)
8 to 10 years	0.69 (0.30-1.61)	2.67 (0.66-10.8)	1.21 (0.51-3.12)	0.52 (0.26-1.05)	0.28 (0.17-0.44)
> 11 years	1	1	1	1	1
Skin color					
White	1.43 (0.80-2.54)	3.14 (0.41-23.7)	1.10 (0.60-2.00)	1.56 (0.89-2.72)	1.19 (0.77-1.87)
Non-white	1	1	1	1	1
Parity					
0	1.54 (0.58-4.13)	0.91 (0.11-7.37)	2.64 (0.80-8.69)	1.74 (0.66-4.63)	1.79 (0.89-3.61)
1 to 2	0.51 (0.33-0.79)	1.37 (0.54-3.48)	0.56 (0.36-0.86)	1.42 (0.93-2.19)	1.05 (0.75-1.46)
> 3	1	1	1	1	1
Obstetric risk					
Usual (low risk)	1	1	1	1	1
Unusual (high risk)	0.67 (0.42-1.06)	1.37 (0.50-3.80)	0.73 (0.46-1.15)	2.09 (1.36-3.20)	1.46 (1.05-2.04)
	ORa (95CI)	**ORa (95CI)**	**ORa (95CI)**	**ORa (95CI)**	**ORa (95CI)**
Funding source					
Private				5.89 (3.11-11.1)	4.27 (2.44-7.47)
Public				1	1
Location					
State Capital	0.57 (0.38-0.85)				0.53 (0.36-0.80)
Countryside	1				1
Age					
10 to 19		16.28 (1.75-151)			
20 to 34		2.30 (0.73-7.31)			
> 35		1			
Years of education					
0 to 7 years					0.22 (0.12-0.39)
8 to 10 years					0.43 (0.25-0.73)
> 11 years					1
Parity					
0	1.48 (0.54-4.05)		2.64 (0.80-8.69)		
1 to 2	0.52 (0.34-0.81)		0.56 (0.36-0.86)		
> 3	1		1		
Obstetric risk					
Usual (low risk)				1	1
Unusual (high risk)				2.24 (1.44-3.47)	1.90 (1.31-2.74)

OR: crude odds ratio; ORa: adjusted odds ratio; 95CI: 95% confidence interval.

vate system and with greater lengths of formal education to maternal choice, since many times the procedure is performed without a medical indication.[23] However, a study conducted in Brazil with 1,136 women showed that 70 to 80% of pregnant women in both health systems (public and private) reported their preference at the beginning of prenatal care for vaginal delivery, which implies that high rates of cesarean sections do not seem to reflect a maternal option.[24]

Labor analgesia is a resource that can minimize the fear of pain that some women have and reduce the indication for cesarean section at the request of the pregnant woman. The National Directive for Normal Labor Care recommends that the patient receive guidance during her prenatal care about the potential risks related to analgesia, namely a longer duration of the second period of delivery and a greater chance of instrumental vaginal delivery. The maternal request would be sufficient and an indication for it to be performed, irrespective of the stage of labor and the degree of dilation.[2] A randomized study with patients receiving regional analgesia versus non-pharmacological measures for pain relief showed a greater reduction in pain scores and satisfaction with the analgesic technique and delivery in the group that was given regional analgesia.[25] The percentage of patients receiving analgesia in our study was higher in parturients in the private health care system, despite it being a universal right. The large numbers of births, small number of anesthetists and procedure rooms in public institutions can justify such inequality.

The current legislation in Brazil determines that health care services allow the presence of an accompanying person, someone to be indicated by the parturient, during labor, delivery and immediate postpartum.[13] The presence of an accompanying person is correlated to the minimization of feelings of loneliness and pain, which allows for a positive parturition experience.[26] However, despite its benefits and legal support, we observe that it does not occur on a regular and systematic basis. In our study, the accompanying person was present only in 40.6% of deliveries, more frequently with women receiving care in the private health care system, in the capital, with a higher education level and lower parity. Data from the "Birth in Brazil" study allowed for finding similar results as independent predictors of having no accompanying person: lower income and education, brown skin color, being a user of the public health care system, multiparity and vaginal delivery.[26] This demonstrates the need to raise awareness about this right and its importance by professionals providing prenatal care in the public health care system, in order for all parturients to benefit from it.

Although there is evidence that the routine use of episiotomy does not present short- or long-term benefits for parturients when compared to its restrictive use,[2,10] the frequency of this intervention was 40.6%, well above the 10% recommended by the WHO[4], and more frequent among younger women. The frequency of episiotomy in primiparous women was 71.4%, but, given their small number, there was no significance following variable adjustment. A cross-sectional study carried out in Spain with 12,093 women also showed an episiotomy rate as high as 50%. The variables that presented a significant association were primiparity (RR=2.98), gestational age greater than 41 weeks (RR=1.2), induced labor (RR=1.33), analgesia (RR=1.95), use of oxytocin (RR=1.58), delivery in the lithotomy position (RR=6.4) and instrumental delivery (RR=1.84).[27]

In a teaching-maternity hospital in Recife, in Brazil's northeast, where the adoption of good practices is systematic, there was a reduction in episiotomy rates from 29% in 2010[28] to 10% in 2014.[29] A case-control study undertaken at this institution showed that the factors most frequently associated with this procedure were physician-assisted births, primiparity, and instrumental births.[29] We may thus conclude that continued medical education, inclusion of the nursing staff in childbirth care, adoption of good practices, and reduction of interventions during labor and delivery result in a reduced number of episiotomies being performed.

There is insufficient evidence to support the routine use of a partogram to manage labor.[9,30] However, considering that some studies have shown benefits from using them, particularly at institutions with fewer resources,[30] the National Directive for Normal Labor Care recommends using the model with a 4h-action line routine.[2] In our study, the more frequent use of a partogram and eating being allowed during labor among women with high-risk pregnancies can be explained by the fact that there is only a single high-risk maternity hospital in the state of Sergipe, which also serves as a center for medical residency and obstetric nursing services, where good care practices are more frequent.

A qualitative, exploratory and descriptive study carried out at a maternity hospital in southern Brazil showed that some practices that are detrimental to the patients' health are still used by professionals who perpetuate previously learned, inadequate models. Such models are expected to make the moment of delivery easier, but without evidence-based justifications for adopting them.[31]

Because this is a cross-sectional study, we could not conduct a time-trend analysis. Nevertheless, since it included a representative number of patients from the

entire state, this is an adequate overview of what happens in Sergipe. Studies that can prospectively evaluate pregnant women during pregnancy until delivery could be more elucidative. Those who can assess the motivations of the agents involved can also identify the reasons for the disparities in care.

In conclusion, in the state of Sergipe, the factors most frequently associated with cesarean section were receiving care in the private health care system, having a greater length of education and having a high-risk pregnancy. Patients in the public health care system and with a lower education level have less access to labor analgesia and to the presence of an accompanying person. It is observed that obstetrical good practices are not universally used and unnecessary interventions, which are still frequent, should be discouraged.

Changes in paradigms, in general, occur gradually. Obstetric care in Brazil remains, in several places, based on "tradition". The importance of encouraging the adoption of scientific, evidence-based protocols in all maternity hospitals, continuing medical education and conducting audit of health care services becomes clear. Accordingly, we suggest that studies evaluating the satisfaction degree of puerperal women with the care received be conducted, comparing different practices that may serve as a stimulus for implementing the necessary changes in behavior.

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Conflict of interest

The authors declare no conflict of interest.

Resumo

Práticas e intervenções obstétricas em mulheres de um estado do Nordeste brasileiro

Objetivo: Descrever as práticas e intervenções utilizadas durante o trabalho de parto e o parto e fatores associados em puérperas do estado de Sergipe.

Método: Estudo transversal com 768 puérperas das 11 maternidades do estado com entrevista após 6h do parto e dados do prontuário da puérpera e dos recém-nascidos. As associações entre as boas práticas e intervenções utilizadas durante o trabalho de parto e o parto com as variáveis de exposição foram descritas em frequências simples, percentuais, razões de chances brutas e ajustadas (ORa) com o intervalo de confiança.

Resultados: Das mulheres estudadas, 10,6% receberam alimentos e 27,8% movimentaram-se durante o trabalho de parto; medidas não farmacológicas para alívio da dor foram realizadas em 26,1%; o partograma estava preenchido em 39,4% dos prontuários; o acompanhante esteve presente em 40,6% dos partos. O uso de ocitocina, amniotomia e analgesia ocorreram em 59,1%, 49,3% e 4,2% das mulheres, respectivamente. O parto ocorreu na posição de litotomia em 95,2% dos casos, houve episiotomia em 43,9% e manobra de Kristeller em 31,7%. Os fatores mais associados à cesariana foram ser usuárias do setor privado de saúde (ORa=4,27; 95CI 2,44-7,47), ter maior escolaridade (ORa=4,54; 95CI 2,56-8,3) e alto risco obstétrico (ORa=1,9; 95CI 1,31-2,74). Usuárias do setor privado tiveram maior presença do acompanhante (ORa=2,12; 95CI 1,18-3,79) e analgesia (ORa=4,96; 95CI 1,7-14,5).

Conclusão: Boas práticas obstétricas são pouco utilizadas e intervenções desnecessárias são frequentes, e os fatores mais associados à cesariana foram ser usuária do setor privado de saúde, ter maior escolaridade e alto risco obstétrico.

Palavras-chave: saúde materno-infantil, trabalho de parto, parto obstétrico, episiotomia, cesárea.

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Correlation between GDF-15 gene polymorphism and the formation of collateral circulation in acute ST-elevation myocardial infarction

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Summary

Objective: To explore the correlation between growth differentiation factor 15 (GDF-15) -3148C/G polymorphism and the formation of collateral circulation in acute ST-elevation myocardial infarction (STEMI) in Han population of Taiyuan area.

Method: The present study included 92 STEMI patients and 56 normal controls based on coronary angiography; STEMI group was divided into collateral group and non-collateral group according to Rentrop's grading method. Polymerase chain reaction (PCR) and DNA sequencing methods were used to detect and analyze the GDF-15 -3148C/G polymorphism in all participants.

Results: There was significant difference in GDF-15 -3148C/G CC and GC distribution between STEMI group and control group (p=0.009); the allele frequencies between these two groups were also significant different (p=0.016); and the risk genotype for STEMI was CC with increased OR=2.660. For STEMI group, GDF-15 -3148C/G CC and GC distribution was also significantly different between patients with and without collateral (p=0.048), and CC genotype significantly promote the formation of collateral circulation. However, there were no significant differences in allele frequencies between these two subgroups of STEMI.

Conclusion: There was correlation between GDF-15-3148C/G polymorphism and the formation of collateral circulation in patients with acute STEMI.

Keywords: collateral circulation, polymorphism, genetic, growth differentiation factor 15, ST elevation myocardial infarction.

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Introduction

When the coronary artery is stenotic or occluded, the formation of collateral circulation is an alternative source of blood supply to the myocardium, which may play an important role in reducing sudden cardiac death and infarct size. Therefore, the presence of coronary collaterals is crucial during acute myocardial infarction. However, significant differences exist in the degree of collateral development among different patients.[1] It is very necessary to find out why some patients can develop sufficient collateral circulation while others do not.

Growth differentiation factor 15 (GDF-15) is a member of the transforming growth factor β (TGF-β) superfamily, which primarily regulates multiple cellular functions as well as the growth of multiple organs and the differentiation and renovation of tissues.[2] The role of GDF-15 in suppressing the progress, invasiveness and metastasis of tumors has been verified by researchers.[3] Besides, it is also related to cardiovascular diseases, including cardiac hypertrophy[4] and heart failure.[5] Recently, GDF-15 +157 A/T polymorphism was found to be associated with coronary collateral formation in acute non-ST segment elevation myocardial infarction.[6] Since GDF-15 -3148C/G polymorphism is closely related to left ventricular remodeling,[7] whether it also plays a role in the formation of collateral vessels in the cardiovascular system remains unclear. The present study aimed to investigate the correlation between GDF-15 -3148C/G polymorphism and the formation of collateral circulation in acute ST-elevation myocardial infarction (STEMI) in the Han population of the Taiyuan area.

Method

Participants

From January 2012 to July 2016, we recruited 92 STEMI patients based on coronary angiography in Taiyuan Central

Hospital, who were allocated into a collateral group and a non-collateral group according to Rentrop's grading method. Fifty-six (56) patients with chest pain but with normal coronary angiography were enrolled in the control group. We excluded patients with the following conditions: Non-ST-elevation myocardial infarction (NSTEMI), acute and chronic inflammatory disease, neoplastic disease, valvular disease, cardiomyopathy, angina pectoris without fixed coronary artery stenosis, X syndrome, coronary artery expansion, severe kidney disease (blood creatinine > 2.5 mg/dL) and severe liver disease (ALT or AST two times higher than normal). Standardized forms were used to collect baseline data of all participants. All participants provided written informed consent.

Coronary angiography and collateral vessels evaluation

Selective coronary angiography was used according to the Judkins method. Any major coronary artery (left main coronary artery and right anterior descending coronary artery, cyclotron branch, the main diagonal branch or blunt edge branch) with pipe cavity diameter stenosis 50% and above was defined as significant coronary artery stenosis.

Using Rentrop's classification system to evaluate the collateral circulation. Level 0: no visible collateral vessel perfusion; Level 1: visible collateral blood vessels, but did not reach the contrast infusion; Level 2: visible collateral blood vessels, epicardial artery perfusion; Level 3: visible collateral blood vessels, epicardial artery was completely perfused. Rentrop's grade zero level is non-collateral group whereas Rentrop's grade 1 ~ 3 level for collateral group.

DNA extraction

DNA was extracted from 3 mL of fasting venous blood in anticoagulation tube using the whole blood genomic DNA extraction kit (Beijing Bao Lai technology co, LTD).

Polymerase chain reaction (PCR) and product identification

Primers were synthesized by Shanghai biological technology co., LTD. 3148C/G site: Forward 5 ´ - AGTGAGTCCTTGTGTCTCTTAC - 3 ´; Reverse : 5 ´ - GCAGGCTGGTGTAGAGTC - 3 ´. Amplification system: a total of 40 μL volume, 10×Buffer 4 μL, $MgCl_2$ 3 μL, dNTP (2.5 mM) 4 μL, forward (10 μM) 2 μL, reverse (10 μM) 2 μL, Taq enzyme (1 U) 2 μL, DNA 2 μL, ddH_2O 21 μL. Polymerase chain reaction (PCR) conditions: 94C° 5 min; 94°C 30 s, 55°C 30 s, 72°C 30 s, repeat 35 cycle; 72°C 7 min. Take 5 μL PCR amplification products with load sample buffer, add sample to 1.5% agarose gel electrophoresis; the gel imaging electrophoresis products were used for analyzing.

Enzyme digestion and product identification

The PCR products were digested for 12 hours (65°C). Reaction system: a total of 20 μL volume, PCR products 10 μL, 10×Buffer 2 μL, enzyme (BsrI enzyme) 0.5 μL. Take 15 μL enzyme-digested products with load sample buffer, add sample to 2.5% agarose gel electrophoresis, the gel imaging electrophoresis products were used for analyzing.

DNA sequence alignment

DNAMAN and Chromas biological software were used to analyze sequencing results.

Statistical methods

SPSS 19.0 was used for statistical analysis. Measurement data were presented as mean ± standard deviation (Mean±SD) and compared by t test; use X^2 test to compare genotype distribution in compliance with Hardy Weinberg genetic balance law and inter-group gene frequencies and alleles. p-value < 0.05 was considered statistically significant.

Results

There was no any statistical significance between three groups in the baseline data (age, gender, smoking history, hyperlipidemia, hypertension, diabetes, family history) (all p>0.05) (Table 1).

As shown in Figure 1, both the size of GDF-15 gene PCR amplification product and CC genotype enzyme digestion product was 251 bp, while GC genotype enzyme digestion product contained three components, whose sizes were 251 bp, 191 bp and 60 bp, respectively. Both CC genotype and GC genotype were observed in all three groups.

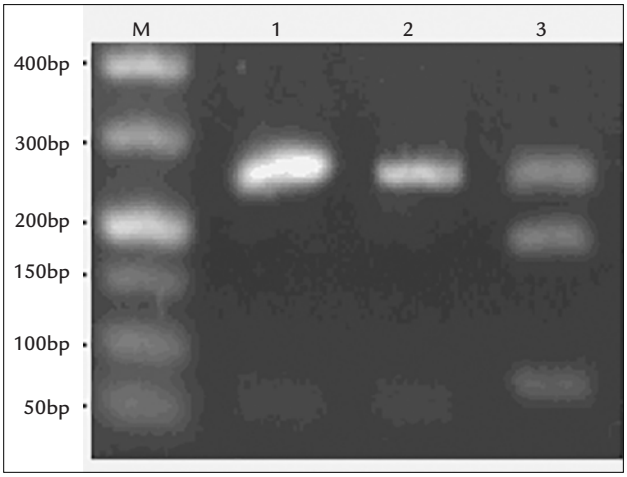

FIGURE 1 -3148C/G agarose gel electrophoresis. 1: PCR amplification products; 2: CC genotype; 3: GC genotype (M is DL500 DNA marker).

TABLE 1 Baseline data comparison (means±SD or absolute numbers).

Survey index	STEMI group (n=92)		Control group (n=56)	p
	Collateral circulation group (n=68)	Non-collateral circulation group (n=24)		
Age (years)	59.15±1.58	59.73±1.63	60.73±1.71	0.453
Gender (n)				0.283
Male	50	15	34	
Female	18	9	22	
Smoking history (n)				0.388
Yes	36	16	35	
No	32	8	21	
Hypertension (n)				0.148
Yes	38	18	29	
No	30	6	27	
Hyperlipidemia (n)				0.361
Yes	39	13	25	
No	29	11	31	
Diabetes (n)				0.164
Yes	32	10	17	
No	36	14	39	
Family history of CHD (n)				0.363
Yes	28	7	17	
No	40	17	39	

STEMI: ST-elevation myocardial infarction; CHD: coronary heart disease.

The results of compared sequences were the same as the enzyme digestion results. Both CC genotype and GC genotype were observed in the STEMI collateral circulation group (Figure 2A), STEMI non-collateral circulation group (Figure 2B) and normal control group (Figure 2C).

The comparison of genotype frequency and allele frequency of GDF-15 -3148C/G site between the STEMI and control groups were presented in Table 2. There was statistically significant difference in -3148C/G site genotype distribution (X^2=6.864, p=0.009) between the two groups. Allele frequency between the two groups also had statistical significance (p=0.016). The risk genotype for STEMI was CC with increased OR=2.660.

The comparison of genotype frequency and allele frequency of GDF-15 -3148C/G locus between the collateral circulation and non-collateral circulation groups was shown in Table 3. There was statistically significant difference in -3148C/G site genotype distribution (p=0.048) between the two groups. However, the allele frequency (p=0.062) between the two groups had no statistical significance. GDF-15 -3148C/G site CC genotype might promote the formation of collateral circulation in patients with STEMI (OR=2.900).

Discussion

When the original coronary artery cannot provide enough blood flow, the collateral circulation has the potential to become the main blood supply, which can reduce sudden cardiac death and infarct size.[8] As we all known, many factors may be associated with collateral development, including severity of coronary stenosis, history of myocardial infarction, use of angiotensin-converting enzyme inhibitors.[9] However, none of the above reasons seems to explain an interesting phenomenon: some patients can develop sufficient collateral circulation while others do not. Therefore, we need to find new influencing factors.

GDF-15 is a member of the transforming growth factor β (TGF-β) superfamily. The role of GDF-15 in cardiovascular disease has been explored by many researchers in recent years. In 2002, Brown et al. reported that serum GDF-15 protein level was an independent risk factor for women's atherosclerosis and other cardiovascular events. It was the first report that connected GDF-15 and cardiovascular disease.[10] Then, the correlations of GDF-15 with cardiac hypertrophy,[4] heart failure[5] and coronary heart disease (CHD)[11] were found gradually. A recently published article reviewed the association of

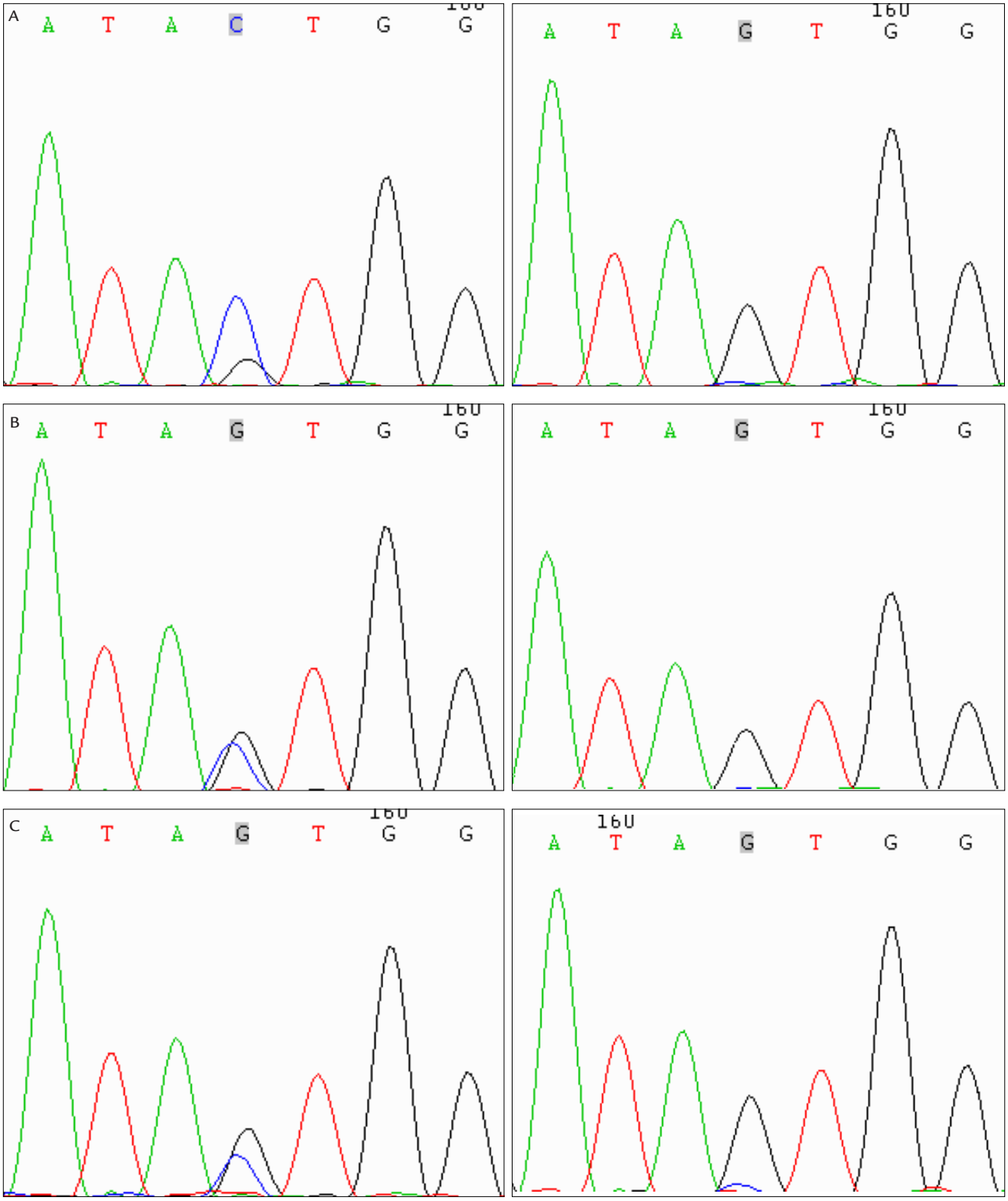

FIGURE 2 A. -3148 site genotype in STEMI collateral circulation group. B. -3148 site genotype in STEMI non-collateral circulation group. C. -3148 site genotype in normal control group (a: GC genotype; b: CC genotype).

TABLE 2 Comparison of genotype frequency and allele frequency of GDF-15 -3148C/G site between the STEMI and control groups (X^2 analysis).

Group	Case	Genotype		Allele frequency	
		CC	GC	C	G
STEMI	92	74 (80.43%)	18 (19.57%)	166 (90.22%)	18 (9.78%)
Control	56	34 (60.71%)	22 (39.29%)	90 (80.36%)	22 (19.64%)
X^2value		6.864		5.792	
p-value		0.009		0.016	
OR value		2.660		2.254	
95CI		1.265-5.595		1.149-4.422	

TABLE 3 Comparison of genotype frequency and allele frequency of GDF-15 -3148C/G site between the collateral circulation and non-collateral circulation groups (X^2 analysis).

Group	Case	Genotype		Allele frequency	
		CC	GC	C	G
Collateral circulation	68	58 (85.29%)	10 (14.71%)	126 (92.65%)	10 (7.35%)
Non-collateral circulation	24	16 (66.67%)	8 (33.33%)	40 (83.33%)	8 (16.67%)
X^2 value		3.911		3.487	
p-value		0.048		0.062	
OR value		2.900		2.520	
95CI		0.983-8.556		0.931-6.819	

GDF-15 with the prognosis of acute coronary syndrome (ACS), finding that high plasma GDF-15 levels were associated with an increased risk of mortality and recurrent myocardial infarction in patients with ACS.[12] All of the studies above indicated the vital role of GDF-15 in cardiovascular disease. Interestingly, in 2010, Sun et al. found that GDF-15 levels increased with the extent of collateral formation;[13] however, the underlying mechanism was not clarified. Recently, Jing et al. observed that there was correlation between GDF-15 + 157 A/T polymorphism and the formation of collateral circulation in patients with non-ST segment elevation myocardial infarction,[6] firstly connecting GDF-15 gene polymorphism with the formation of coronary collateral circulation. Apart from the + 157 A/T site, the relation between -3148C/G (rs4808793) polymorphism and cardiovascular disease was also investigated. One study failed to prove an association of - 3148C/G polymorphism with CAD or its severity in a Chinese population.[14] Conversely, the other research suggested that GDF-15 -3148C/G polymorphism was closely related to left ventricular remodeling.[7] Therefore, whether GDF-15 -3148C/G polymorphism also plays a role in the formation of coronary collateral vessels or not remains unclear and needs to be further clarified.

In the present study, we included 92 STEMI patients and 56 normal controls based on coronary angiography; the STEMI group was divided into a collateral group and a non-collateral group. Two genotypes of -3148C/G sites, CC and GC, were found both in the STEMI group and the control group, meaning that GDF-15 -3148C/G polymorphism existed in the Han population of the Taiyuan area. There was significantly difference in the distribution of these two genotypes between the STEMI group and the control group (p=0.009). Allele frequencies between these two groups were also significantly different (p=0.016). Moreover, CC genotype significantly increased the risk of STEMI occurrence (OR=2.660). At the same time, the possibility of the existence of collateral circulation in patients with STEMI carrying CC genotype could be increased by 2.9 times. However, there were no significant differences in allele frequencies between the two subgroups of STEMI; this may be due to the small sample size of this study. We need larger samples in the future researches.

Most importantly, we found that GDF-15 -3148C/G polymorphism (CC genotype) might have a correlation with STEMI occurrence and the formation of collateral circulation. Even though one study suggested that GDF-15 might predict more severe coronary stenosis, which had a higher probability to develop collaterals,[13] the specific mechanism of action is still unknown, further functional studies are thus needed to clarify it. Of course, our study has some limitations: first, this is a cross-sectional

study and does not provide a mechanism explaining the results; second, the study sample might be considered small, which limits the reliability of our results; third, our results may not be applicable to the Chinese population as a whole. More longitudinal studies and functional studies in different ethnicities are needed to further investigate the pathophysiological effects of GDF-15.

Conflict of interest

The authors declare no conflict of interest.

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Obstructive sleep apnea syndrome and sleep quality in hypertensive patients

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Summary

Introduction: Obstructive sleep apnea and hypopnea syndrome (OSAHS) is one of the developmental factors of high blood pressure (HBP), a relevant global public health problem. OSAHS is characterized by the reduction or complete cessation of respiratory airflow due to intermittent airway collapse. Additionally, significant changes in sleep rhythm and pattern are observed in these patients.
Objective: To evaluate the association between OSAHS and sleep quality in essential and resistant hypertensives.
Method: A cross-sectional, observational study evaluated 43 hypertensive patients treated at the outpatient clinics of the Faculdade de Medicina do ABC (FMABC) who were medicated with two or more antihypertensive drugs and divided into nonresistant or resistant to treatment.
Results: Group I (using up to two antihypertensive agents – 60.47% of the sample) presented mean systolic blood pressure (SBP) of 127.5±6.4 mmHg, mean diastolic blood pressure (DBP) of 79.6±5.2 mmHg, mean body mass index (BMI) of 27.2±5.3 kg/m^2 and mean age of 51.2±15.1 years. Group II (using more than two antihypertensive drugs – 37.2% of the sample) presented mean SBP of 132.1±9.3 mmHg, mean DBP of 84.5±5.8 mmHg, mean BMI of 27.2±7.2 kg/m^2 and mean age of 55.5±13.4 years. The patients presented low quality of sleep/sleep disorder evaluated by the Pittsburgh Sleep Quality Index (PSQI), which represents a preponderant factor for OSAHS.
Conclusion: Patients at high risk for OSAHS had poor sleep quality and high levels of DBP, suggesting a causal relation between these parameters. However, they did not present a higher prevalence of resistant high blood pressure (RHBP).

Keywords: apnea, sleep apnea syndromes, sleep apnea, obstructive, hypertension, sleep.

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Introduction

High blood pressure (HBP) has a high prevalence in our country and a low control rate; it is a multifactorial clinical condition characterized by elevated and sustained blood pressure (BP) levels.[1] It is one of the main modifiable risk factors and the most important public health problem.[2] It is estimated that 12-15% of hypertensive patients have resistant high blood pressure (RHBP).[3] RHBP is characterized as a difficult-to-manage clinical condition, associated with high cardiovascular risk. Pseudo-resistant HBP is mistaken for true RHBP due to non-adherence to treatment, inadequate blood pressure measurements, inappropriate therapeutic doses or regimens, in addition to the white coat effect.[4,5] Most people with RHBP have advanced age, obesity, left ventricular hypertrophy, diabetes mellitus, chronic nephropathy, metabolic syndrome, an increased salt intake, are from African descent and less physically active. There are several causal factors for RHBP, with obstructive sleep apnea and hypopnea syndrome (OSAHS) being the most common causes.[6,7] OSAHS is characterized as the reduction in or complete cessation of respiratory airflow due to intermittent airway collapse,

despite sustained respiratory effort.[8] During OSAHS events, the individual presents with hypoxemia, exacerbated snoring, and recurrent micro-arousals that restore respiration and blood oxygenation.

Snoring and micro-awakenings disrupt sleep, favoring the onset of classic symptoms such as excessive daytime sleepiness, sensation of unrefreshing sleep, reduced ability to concentrate and fatigue.[8] The apnea-hypopnea index (AHI), i.e. the mean number of apnea and hypoxia events that occur per hour, is used to assess disease severity. OSAHS is defined as AHI ≥ 5 and is associated with excessive daytime sleepiness.[9] The prevalence of OSAHS, as defined by an AHI ≥ 5 and resulting from the analysis of eleven epidemiological studies published between 1993 and 2013, averaged 22% (range 9-37%) in males and 17% (range 4-50%) in females, while excessive daytime sleepiness occurred in 6% (range 3-18%) of males and in 4% (range 1-17%) of females. OSAHS is more prevalent in males and increases with age and obesity.[10] In Brazil, there are no comprehensive studies, but the prevalence of OSAHS can be estimated in a cross-sectional study of a population sample from São Paulo. This study analyzed volunteers aged between 20 and 80 years, diagnosed with OSAHS by means of polysomnography, a gold standard for diagnosing this condition. Of 1,042 individuals selected, 55% were male and 60% had a body mass index (BMI) greater than 25 kg/m^2. OSAHS was diagnosed in 32.8% of individuals (95CI 29.6-36.3). The independent, OSAHS-associated factors were identified as being male (OR=4.1; 95CI 2.9-5.8; p<0.001), obesity (OR=10.5; 95CI 7.1-15.7; p<0.001) and age greater than 60 years (OR=34.5; 95CI 18.5-64.2; p<0.001).[11] In each obstructive apnea-hypopnea event, forced inspiration against the occluded airway is accompanied by negative intra-pleural pressure. As apnea prolongs, hypoxemia and hypercapnia intensify, thereby promoting pulmonary vasoconstriction, which leads to the development of transient pulmonary hypertension. Simultaneously, there is stimulation of the sympathetic nervous system, with systemic vasoconstriction and HBP, maintaining a high level of systolic blood pressure (SBP) after an apnea event, even in individuals whose blood pressure levels are normal during wakefulness.[12] In addition, the phenomenon of hypoxemia followed by subsequent reoxygenation, which repeats over and over several times during the night, cause changes in reperfusion and leads to the formation of free radicals,[13] oxidative stress, homocysteine and cysteine, which in turn are determining contributors to cardiovascular changes.[14] An association between OSAHS and obesity, its predominance in males and in menopaused women, as well as the systemic effects triggered by its onset, strongly suggest that OSAHS is a systemic disease and not a local abnormality. Innumerable other factors are likely involved in the lesions observed in these patients,[9,10] which, in the long term, promote chronic alterations in the cardiovascular system. Polysomnography, the gold standard for its diagnosis, is a high-cost test, which contributes to other diagnostic alternatives being used instead.[12,13] Administering international questionnaires can estimate and predict the severity of sleep disorders by assessing excessive daytime sleepiness with the Epworth Sleepiness Scale (ESS), sleep quality with the Pittsburgh Sleep Quality Index (PSQI), and risk of OSAHS with the Berlin Questionnaire (BQ).[14-16]

The objective of our study was to evaluate the association between OSAHS and sleep quality in patients with essential and resistant hypertension.

Method

This is an observational, cross-sectional study with high-blood-pressure patients from the outpatient clinic at Faculdade de Medicina do ABC (FMABC), who were treated with either two antihypertensive drugs (nonresistant) or more than two antihypertensive drugs (resistant).

Patients with a high blood pressure diagnosis and aged over 18 years were considered eligible to participate in the study provided they met the criteria set forth by the VI Brazilian Guidelines on Hypertension of the Brazilian Society of Cardiology. Those not adhering to the antihypertensive treatment were excluded from the study according to Morisky test,[17,18] as were those affected by the white coat effect.

We used the Pittsburgh Sleep Quality Index (PSQI), comprising ten questions, to assess sleep quality. Questions number one, two, three and four are open-answer questions; whereas questions from five to ten are objective, with questions five, nine and ten having a blank space for the interviewee's comments. The PSQI questions make up seven components, which are then analyzed based on the scoring instructions for each of these components, ranging from 0 to 3. This instrument's maximum overall score is 21, with scores higher than 5 indicating poor sleep quality.[16] This index has a sensitivity of 80%, and its specificity is 68.8%. We used the Epworth Sleepiness Scale (ESS) to assess the degree of daytime sleepiness. The scale consists of eight daily-life situations where the patient is asked to score between 0 and 3 their chance of napping when performing daily activities, with the following meaning: 0, no chance of dozing off; 1, little chance of dozing off; 2, a fair chance of dozing off; and 3, a high chance of

dozing off. The scale can range from 0 to 24. Scores above 10 denote excessive daytime sleepiness.[19] In order to track the risk of OSAHS, we used the BQ, which includes 10 items, organized into three categories relative to snoring and apnea (5 items), daytime sleepiness (4 items) with a sub-question on sleepiness while driving (nap episodes at the steering wheel, while driving a motor vehicle), and high blood pressure or obesity (1 item). The risk-based classification (high risk versus non-high risk) relied on the responses for each category. Category I – persistent symptoms (> 3-4 times/week) on two or more questions. Category II – persistent symptoms (> 3-4 times/week), when excessive daytime sleepiness, sleepiness while driving a motorized car or both were reported. Category III – patient with a personal history of HBP or a BMI greater than 30 kg/m^2. Those who scored in at least two categories were considered at high risk.[20]

To the selected patients, we administered the Berlin, ESS and the PSQI questionnaires to assess sleep quality, OSAHS and daytime sleepiness. To assess adherence to medication intake, patients underwent the Morisky-Green Test. In addition to the questionnaires, we took ambulatory blood pressure measurement in accordance with the parameters set by the VI Brazilian Hypertension Guidelines of the Brazilian Society of Cardiology. In order to describe the characteristics studied, we used absolute and relative frequencies. We used the Chi-squared test to evaluate the association between these characteristics and the risk of OSAHS with the Berlin questionnaire. The confidence level was 95%. The software we used was Stata 11.0.

Results

Of the 56 interviewees, 13 displayed poor adherence to medication intake and were thus excluded from the study. Of the remaining 43 patients, 62.8% were Caucasian women (62.8%) displaying a high adherence rate (72.1%), most of whom did not report good sleep quality (65.11%). Other characteristics of the population studied are shown in Table 1.

The Berlin questionnaire, which we administered in order to assess the risk of OSAHS (Table 2), showed a statistically significant association between poor sleep quality/sleep disturbance, assessed with PSQI, and a high risk of OSAHS ($p<0.037$).

The 43 patients were divided into two groups: Group I – those who used up to two medications for treating HBP, representing 60.47% of the sample (26), and had a mean SBP of 127.5±6.4 mmHg, a mean DBP of 79.6±5.2 mmHg, a mean BMI of 27.2±5.3 kg/m^2 and a mean age of 51.2±15.1 years. Group II – those who used more than two medications, totaling 37.2% of the sample (17), had a mean SBP of 132.1±9.3 mmHg, mean DBP of 84.5±5.8 mmHg, mean BMI of 27.2±7.2 kg/m^2 and mean age of 55.5±13.4 years. There was no statistically significant correlation between amount of medication and poor sleep quality ($p=0.4803$). There was statistically significant correlation between a high level of DBP and the risk of OSAHS (Table 3).

TABLE 1 Characterization of study variables.

Characteristics	n	%
Sex		
Female	26	62.8
Male	17	37.2
Ethnicity		
Caucasian	27	62.8
Non-caucasian	16	37.2
Epworth Questionnaire		
Normal daytime sleepiness	34	79.1
Excessive daytime sleepiness	9	20.9
Berlin Questionnaire		
Low risk for OSAHS	18	41.9
High risk for OSAHS	25	58.1
Pittsburgh Sleep Quality Index		
Sleep quality		
Good	15	34.9
Bad	13	30.2
Sleep disorder	15	34.9
Diabetes mellitus		
Yes	8	18.6
No	35	81.4
Chronic kidney disease		
Yes	19	44.2
No	24	55.2
Medication adherence		
High	31	72.1
Moderate	12	27.9
BMI (kg/m^2)		
< 25	16	37.2
25-30	14	32.6
> 30	13	30.2
	Mean±SD	
Age (years)	52.9±14.5	14.5
BMI (kg/m^2)	27.2±6.0	6.0
SBP (mmHg)	129.3±7.9	7.9
DBP (mmHg)	81.6±5.9	5.9

OSAHS: obstructive sleep apnea and hypopnea syndrome; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

TABLE 2 Association of characteristics of study variables according to the Berlin Questionnaire.

Characteristics	Berlin (risk for OSAHS)		p*
	Low risk	High risk	
	n (%)		
Medication adherence			0.9872
High	13 (72.2)	18 (72)	
Moderate	5 (27.8)	6 (28)	
Epworth Questionnaire			1.0000
Normal daytime sleepiness	14 (77.8)	20 (80)	
Excessive daytime sleepiness	4 (22.2)	5 (20)	
PSQI			0.0037
Good sleep quality	11 (61.1)	4 (16)	
Bad sleep quality	5 (27.8)	8 (32)	
Sleep disorder	2 (11.1)	13 (52)	
Ethnicity			1.0000
Caucasian	11 (61.1)	16 (64)	
African descent	6 (33.3)	8 (32)	
Asian	1 (5.6)	1 (4)	
Sex			0.6555
Female	12 (66.7)	15 (60)	
Male	6 (33.3)	10 (40)	
CKD			0.9769
No	10 (55.6)	14 (56)	
Yes	8 (44.4)	11 (44)	
DM			0.7010
No	14 (77.8)	21 (84)	
Yes	4 (22.2)	4 (16)	
BMI (kg/m^2)			0.1649
< 25	8 (44.4)	8 (32)	
25-30	3 (16.7)	11 (44)	
> 30	7 (38.9)	6 (24)	

* Chi-square.
OSAHS: obstructive sleep apnea and hypopnea syndrome; PSQI: Pittsburgh Sleep Quality Index; CKD: chronic kidney disease; DM: diabetes mellitus; BMI: body mass index.

TABLE 3 Association of study groups and means according to the Berlin questionnaire.

	Berlin (risk for OSAHS)		p*
	Low risk	High risk	
	n (%)		
Number of medications			0.4803
2 or less	12 (66.7)	14 (56)	
More than 2	6 (33.3)	11 (44)	
	Mean±SD		
Age (years)	49.3±16.4	55.5±12.7	0.1652
SBP (mmHg)	126.6±6.6	131.4±8.4	0.0493
DBP (mmHg)	79.6±4.9	83±6.2	0.0585
BMI (kg/m^2)	27.2±6.4	27.5±6.0	0.8994

* Chi-square.
OSAHS: obstructive sleep apnea and hypopnea syndrome; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index.

Discussion

The groups were similar to each other with regard to age and BMI, but differed in relation to diastolic pressure levels ($p<0.06$). In our study, we found no statistically significant difference ($p>0.48$) after administering the BQ when correlating high-risk versus low-risk group for OSAHS and the groups using more than two medications (RHBP) and those using two medications at most. In comparing our data with those reported in the literature, we observed a 64-80% incidence of association between RHBP and OSAHS as a secondary cause of RHBP.[11,20,21] We can attribute our findings to confounding factors such as age, sex, height, BMI, cervical circumference, level of education and pseudo-adherence, given that the mean SBP values were 127.5±6.4 mmHg, a mean DBP of 79.6±5.2 mmHg, a mean BMI of 27.2±5.3 kg/m^2 and a mean age of 51.2±15.1 years. In the two-drug group, the mean SBP value was 132.1±9.3 mmHg, the mean DBP was 84.5±5.8 mmHg, the mean BMI was 27.2±7.2 kg/m^2 and the mean age was 55.5±13.4 years, whereas in the group of patients using more than two medications there were no statistically significant differences. These discrepancies between our results and those reported in the literature do not invalidate the BQ's usefulness in screening OSAHS in patients with BP and sleep disorders, since they have a sensitivity of 85.5% and specificity of 65% if we consider a cut-off equal to or greater than 10 for the apnea-hypopnea index.

Our study showed a statistically significant correlation between patients at high risk of OSAHS and poor sleep quality, but no evidence of a correlation between daytime sleepiness and risk of OSAHS, since most of the patients with excessive daytime sleepiness had difficulty understanding the situations described in the questionnaire. A high risk of OSAHS was associated with older age ranges, as well as with higher blood pressure levels, but remained statistically significant with the DBP levels.

Comparing the groups, we found no significant association. Despite the fact that even though patients using more than two drugs had a higher risk of OSAHS, the difference was not statistically significant. In this group, we found higher SBP levels and greater ages; these are factors that, per se, are predictive of a high risk of OSAHS. These data reinforce the relation between HBP and OSAHS: 58.14% of patients with HBP are at a high risk of OSAHS, despite the use of methods less sensitive and specific than polysomnography to assess the existence of obstructive sleep apnea. With respect to RHBP, there should be greater attention from both the general practitioner and the cardiologist, considering that many cases of RHBP are erroneously classified due to low adherence to medication intake. Though sometimes more than three medications are prescribed, the expected decline in pressure levels is not achieved because these patients do not take their medications properly.

Conclusion

Considering our findings, we conclude that patients at a high risk of OSAHS had poorer sleep quality and higher DBP levels. However, they did not show a higher prevalence of RHBP.

Resumo

Síndrome da apneia obstrutiva do sono e qualidade do sono em hipertensos

Introdução: A síndrome da apneia e a hipopneia obstrutiva do sono (SAHOS) estão inseridas entre os fatores de desenvolvimento da hipertensão arterial sistêmica (HAS), um relevante problema de saúde pública mundial. A SAHOS é caracterizada pela redução ou cessação completa do fluxo aéreo respiratório, decorrente do colapso intermitente das vias respiratórias. Adicionalmente, observam-se nos pacientes importantes alterações no ritmo e padrão do sono.
Objetivo: Avaliar a associação entre SAHOS e qualidade de sono em hipertensos essenciais e resistentes.
Método: Estudo observacional, transversal avaliou 43 pacientes hipertensos provenientes dos ambulatórios da Faculdade de Medicina do ABC (FMABC) medicados com dois ou mais anti-hipertensivos, divididos em não resistentes ou resistentes ao tratamento.
Resultados: Grupo I (que utilizava até dois anti-hipertensivos – 60,47% da amostra) apresentou pressão arterial sistêmica (PAS) média de 127,5±6,4 mmHg, pressão arterial diastólica (PAD) média de 79,6±5,2 mmHg, índice de massa corpórea (IMC) médio de 27,2± 5,3 kg/m^2 e idade média de 51,2±15,1 anos. Grupo II (que utilizava mais que dois anti-hipertensivos – 37,2% da amostra) apresentou PAS média de 132,1±9,3 mmHg, PAD média de 84,5±5,8 mmHg, IMC médio de 27,2±7,2 kg/m^2 e idade média de 55,5±13,4 anos. Os pacientes apresentaram baixa qualidade de sono/distúrbio do sono avaliada pelo PSQI, o que representa um fator preponderante para SAHOS.
Conclusão: Os pacientes com alto risco para SAHOS tiveram pior qualidade de sono e elevados níveis de PAD, sugerindo uma relação causal entre esses parâmetros. Contudo, não apresentaram maior prevalência de hipertensão arterial resistente.

Palavras-chave: apneia, síndromes da apneia do sono, apneia obstrutiva do sono, hipertensão, sono.

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The safety and clinical efficacy of recombinant human granulocyte colony stimulating factor injection for colon cancer patients undergoing chemotherapy

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Summary

Objective: The present study was designed to evaluate safety and efficacy of recombinant human granulocyte colony stimulating factor (G-CSF) injection and whether this regimen could reduce the incidence of adverse events caused by chemotherapy.

Method: A total of 100 patients with colon cancer who were treated with chemotherapy in our hospital from January 2011 to December 2014 were randomly divided into two groups, with 50 patients in each group. The patients in the treatment group received G-CSF 24 hours after chemotherapy for consecutive three days; the patients in the control group received the same dose of normal saline. Routine blood tests were performed 7 days and 14 days after chemotherapy.

Results: Compared with the control group, the incidences of febrile neutropenia and leukocytopenia in the treatment group were significantly lower ($p<0.05$). In addition, the incidence of liver dysfunction in the treatment group was lower than that of the control group, without statistical significance. The incidence of myalgia in the treatment was higher than that of the control group without statistical significance.

Conclusion: The present study indicated that G-CSF injection after chemotherapy is safe and effective for preventing adverse events in colon cancer patients with chemotherapy.

Keywords: granulocyte-macrophage colony-stimulating factor, drug therapy, febrile neutropenia.

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Introduction

Although advanced screening and preventative strategies have been widely applied in recent years, colorectal carcinoma remains a major public health problem worldwide.[1] Currently, surgical resection of the primary colorectal lesions combined with adjuvant chemotherapy and radiation is the mainstay of treatment.[2] However, despite recent advances in earlier detection and improvements in chemotherapy, the median survival for all patients with metastatic colorectal carcinoma is approximately 22-24 months, with a 5-year survival rate < 5 %.[3]

Leukopenia is one of the most common adverse events and dosage-limiting toxicities of chemotherapy for patients with colon cancer. In addition, it results in febrile neutropenia (FN) which is related to life-threatening infections with a mortality rate of 7-11%.[4-6] The previous study has shown that granulocyte colony stimulating factor (G-CSF) could decrease the incidence of leukopenia and FN, and, therefore, infections and infection-related mortality, as well as the incidence of chemotherapy dosage reductions and regimen delays.[7] Usually, G-CSF is recommended after chemotherapy until complete recovery of leukocyte levels. Weycker et al.[8] have shown that prophylactic application of G-CSF resulted in a one-third to two-third decrease in the risk of re-hospitalization due to FN in patients treated with chemotherapy. In addition, two more recent clinical

studies[8,9] on comparative effectiveness of G-CSF prophylaxis yielded similar findings. In China, G-CSF has been widely used to reduce chemotherapy-induced leukopenia. However, the clinical efficacy and safety of prophylactic application of G-CSF for Chinese colon cancer patients have not been fully studied yet. Therefore, we designed and conducted the present comparative study. A total of 100 patients with colon cancer who were treated with chemotherapy were randomly divided into two groups, with 50 patients in each group. The treatment group received G-CSF prophylaxis 24 hours after chemotherapy for three consecutive days. The control group received G-CSF with the same amount of normal saline.

Method

Patient eligibility

All of the patients had their diagnosis of metastatic colorectal adenocarcinomas confirmed by pathology and/or cytology. They all underwent chemotherapy in Subei People's Hospital (Yangzhou, Jiangsu, China) from January 2011 to December 2014. Inclusion criteria were: (1) Karnofsky performance status ≥ 60; (2) age between 18-75 years; (3) predicted survival time ≥ 3 months; (4) adequate bone marrow functions indicated by white blood cell counts > 4.0×10^9 and transaminases < 1.5 times and within the upper limit of normal; (5) without heart, liver and kidney dysfunctions. Exclusion criteria were: (1) unability to complete two cycles of chemotherapy; (2) any severe medical and/or psychiatric conditions; (3) presence of any other malignancies; (4) women who were pregnant or breastfeeding at the time of study.

The present study was reviewed and approved by the Medical Ethics Committee of West China Hospital (Chengdu, Sichuan, China). All participants provided written consent forms.

Treatment method

A total of 100 eligible patients were randomly divided into two groups. Patients in the treatment group received subcutaneous administration of G-CSF (JiSaiXin, Huabei Jintan pharmaceutical Co.) 24 hours after chemotherapy, 150 μg per day, for three consecutive days. Patients in the control group received the same amount of normal saline. Routine blood tests were performed 7 days and 14 days after chemotherapy.

Adverse events were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events, v3.0. Dose-limiting toxicity (DLT) was defined as grade 3/4 non-hematologic adverse events and grade 4 hematologic adverse events.

Statistical analysis

IBM SPSS 17.0 statistical software was used to analyze the data. Continuous data were expressed as mean ± standard deviation (SD). Categorical data were expressed as percentages. Intergroup comparisons were performed using Chi-square test (or Fisher's exact test) or variance analysis or rank sum test. All the tests were performed using a two-sided test of difference, where the inspection level α of 0.05 and a difference with $p<0.05$ were considered statistically significant.

Results

Patient characteristics

As shown in Table 1, a total of 100 patients were enrolled in the present study including 50 in the treatment group and 50 in the control group. All patients received at least two cycles of chemotherapy prior to the enrollment. In the treatment group, the age range was between 25 and 77 years with a mean age of 57 years. There were 30 male and 20 female patients. In the control group, the age range was between 27-75 years, with a mean age of 57 years. There were 29 male and 21 female patients.

TABLE 1 General clinical characteristics of patients.

	Treatment group	Control group
Median age (range, years)	57 (25-77)	58 (27-75)
Gender		
Male	30	29
Female	20	21
Treatment		
No prior chemotherapy	37	33
With prior chemotherapy	13	17
Primary tumor site		
Liver cancer	20	19
Lung cancer	16	12
Other	14	19

Adverse events

In the treatment group, there was no patient diagnosed with FN, in other words, an FN incidence of 0%. In the control group, there were three patients diagnosed with FN, yielding an FN incidence of 6%. In addition, in the treatment group, there were 17 patients diagnosed with leukopenia grade I/II and one patient diagnosed with leukopenia grade III/IV with the leukopenia incidence of 36%. In addition, there were 17 patients diagnosed with leukopenia grade I/II and one patient diagnosed with leukopenia grade III/IV with the leukopenia incidence of 36%. In the control group, there were 27 patients diagnosed with leu-

kopenia grade I/II and nine patients diagnosed with leukopenia grade III/IV with a leukopenia incidence of 72%. The incidences of FN and leukopenia were significantly lower in the treatment group compared with that of the control group (both p-values < 0.05). The differences in incidence of liver dysfunction, renal function abnormality, shock, acute interstitial pneumonia and myalgia were not statistically significant between the two groups (all p-values > 0.05). Adverse events were shown in Table 2.

TABLE 2 Adverse events of Chinese colon cancer patients with prior chemotherapy with or without G-CSF administration.

Adverse events	Treatment group	Control group
Febrile neutropenia	0	3
Leukopenia		
Grade I/II	17	27
Grade III/IV	1	9
Myalgia	3	2
Liver dysfunction	7	8
Renal function abnormality	0	0
Shock	0	0
Acute interstitial pneumonia	0	0

Toxicity

No DLTs were observed. Local reactions at injection sites including erythema, induration, and pruritus were observed in all patients, and tenderness in five of 100 patients. Minimal systemic toxicities were observed in the treatment group (Table 3). The most common adverse effects were transient, low-grade fever/chill (14%), fatigue (8%), nausea (6%) and headache (12%). Fever and chills were likely due to G-CSF treatment, while nausea was probably the result of cyclophosphamide administration. Headache may have been the result of ondansetron, which was provided as an optional and prophylactic antiemetic before the administration of cyclophosphamide. Therefore, the dose level we used in the present study was considered to have an acceptable degree of safety and warrants subsequent trials to further assess G-CSF associated clinical outcomes.

TABLE 3 G-CSF-related systemic adverse reactions.

Adverse reactions	CTC grade	No. of events	Incidences of adverse reactions (%)
Fever/chill	1	7	14
Headache	1	6	12
Fatigue	1	4	8
Nausea	1	3	6

CTC: common terminology criteria for adverse reactions.

Discussion

The present study has demonstrated the clinical efficacy and safety of G-CSF for Chinese patients with metastatic colorectal cancer. To our knowledge, this was the first study of a G-CSF for colorectal cancer patients.

As one of the main side-effects of chemotherapy, bone marrow suppression has serious impacts on clinical efficacy and safety for patients with malignancies undergoing chemotherapy. As a specific hematopoietic regulating growth factor of granulocyte lineage, G-CSF can be produced by gene recombination technique and therefore become recombinant human granulocyte colony stimulating factor.[10-12] It promotes the multipotent hemopoietic stem cells to differentiate into mature granulocytes and macrophages in peripheral blood.[13]

Based on the American Society of Clinical Oncology (ASCO) recommendations for the use of white blood cell growth factors in 2006[14] and the National Comprehensive Cancer Network (NCCN)[15] guidelines for the use of myeloid growth factors in 2013, G-CSF's primary prophylactic use, secondary prophylactic use and therapeutic use were stated as follows. Primary prophylactic use was recommended for patients based on the risks of developing FN. Secondary prophylactic use was recommended for patients who had developed neutropenic complications from a prior cycle of chemotherapy. Therapeutic use should be considered for patients with fever and neutropenia who were at high risk of infection-associated complications or who had prognostic factors which predicted poor clinical outcomes. In addition, patient's age, chemoradiotherapy regimen, pneumonia, hypotension, and nutritional status were not factors to determine whether or not influence use G-CSF.

The present study has demonstrated that the incidences of FN and leukopenia were significantly lower in the treatment group compared with that of the control group (both p-values < 0.05), with the incidences of FN being 0% in the treatment group and 6% in the control group, and the incidences of leukopenia being 36% in the treatment group and 72% in the control group. The differences of incidences of liver dysfunction, renal function abnormality, shock, acute interstitial pneumonia and myalgia were not statistically significant between the two groups (all p-values > 0.05).

Although G-CSF alone as an adjuvant therapy showed some promise in colon cancer patients,[16-18] its role as an adjuvant therapy when combined with chemotherapy remains to be determined. The present study described a novel adjuvant therapy for colorectal cancer chemotherapy. The present study found it to be safe and well toler-

ated for Chinese patients with colon cancer undergoing chemotherapy to receive subcutaneously administration of G-CSF 24 hours after chemotherapy 150 μg per day for consecutive three days.

However, there were some limitations in the present study. Firstly, the present study was conducted using relatively small sample size of patients in a single institution. Therefore, multiple-centered studies with larger sample size of penitents will be needed to verify our results and conclusions. Secondly, in the present study, G-CSF administration started 24 hours after chemotherapy and lasted for three consecutive days based on our clinical experience. However, the best regimen for G-CSF administration has been unknown. Therefore, we recommended further studies to verify our results and conclusion and determine the best regimen for G-CSF administration.

Conclusion

In conclusion, our study suggests that prophylactic administration of G-CSF 24 hours after chemotherapy 150 μg per day for three consecutive days is a safe and effective treatment for preventing FN and leukopenia in Chinese patients with colon cancer undergoing chemotherapy.

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Elevated neutrophil-to-lymphocyte ratio in the diagnosis of Hashimoto's thyroiditis

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Summary

Objective: Hashimoto's thyroiditis (HT) is an autoimmune inflammatory disorder. The purpose of this study was to determine the neutrophil-to-lymphocyte ratio (NLR), a novel marker of inflammation, in patients with HT and to compare these values with those from healthy subjects.
Method: A total of 154 participants were included in the study, 90 HT patients and 64 healthy volunteers. Retrospectively, demographic and laboratory data of the subjects were obtained from our institution's database. Patients with active infection, diabetes mellitus, malignancy, other chronic inflammatory diseases, hematologic disorders and patients on aspirin or steroid treatment were excluded from the study. Values for complete blood count (CBC) and serum laboratory parameters of HT patients were the baseline values obtained at the time of HT diagnosis. Control subjects consisted of healthy volunteers who visited our institution for a routine check-up.
Results: Age, gender and CBC parameters were not different between the HT group and the control group; however, the NLR of HT group (2.1 [1.3-5.8]) was significantly higher than the control group (1.9 [0.6-3.3]), p=0.04.
Conclusion: Increased NLR may be useful as an indicator of the presence of HT, especially in complicated cases. NLR is inexpensive and easy to determine. Larger, prospective studies are required to determine its usefulness in assessing diagnostic potential and treatment outcomes in HT patients.

Keywords: Hashimoto disease, blood cell count, inflammation, diagnosis.

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Introduction

Hashimoto's thyroiditis (HT) is the most common autoimmune thyroiditis worldwide, with prevalence as great as 6% in iodine-deficient countries.[1] Typically, the disorder is more frequently seen in women. The characteristic histopathological feature of HT is lymphoplasmacytic inflammation of the thyroid tissue.[2] Circulating autoantibodies against thyroid peroxidase and thyroglobulin are elevated in the serum of patients with HT.

Complete blood count (CBC)-derived parameters and their relation to certain diseases have recently received attention from researchers. One of these CBC parameters is the neutrophil-to-lymphocyte ratio (NLR). NLR is considered to be a marker of inflammation and, due to its simplicity and low cost, has been studied in many medical conditions.[3] An elevated neutrophil count in a CBC predicts ongoing inflammation and decreased lymphocyte count is considered to be an indicator of poor prognosis, so a combination of these two measures is generally accepted to be predictive of an inflammatory situation.[3] NLR reflects both inflammatory burden (by neutrophil count) and regulatory mechanisms (by lymphocyte count) in inflammatory disease.[4,5] Studies suggest NLR is associated with occult inflammation in certain conditions.[6-8] NLR has also been shown to be useful in predicting adverse outcomes in patients with pancreatitis,[9] appendicitis[10] and other critical conditions.[11]

NLR has not previously been studied in HT subjects, and, in this retrospective study, NLR was determined in 90 HT subjects and investigated the possible association

between HT and NLR by comparing these values to NLR determined in a healthy population.

Objective

Since there is a strong association between inflammation and HT, and between inflammation and NLR, we aimed to compare NLR values of patients with HT to those of healthy volunteers.

Method

Patients with diagnosis of HT who were followed up in the internal medicine clinics of our institution were enrolled to present retrospective study. Diagnosis of HT was established with a combination of relevant history and findings in physical examination that were supported by characteristic findings on ultrasound scan (diffuse enlargement of the gland and decreased echo pattern) and elevated serum anti-thyroid peroxidase or anti-thyroglobulin levels. Patients with known active infection, diabetes mellitus, malignancy, other chronic inflammatory diseases and hematologic disorders were not included in the study. Patients on steroid or aspirin treatment were also excluded.

CBC and serum parameters used in this study were the baseline laboratory findings that were recorded in our database at the time of HT diagnosis. Control subjects consisted of healthy volunteers who visited our institution for a routine check-up. General characteristics and laboratory data of all participants were obtained from the computerized database of our clinics. White blood cell count (WBC), neutrophil count (Neu), lymphocyte count (Lym), hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV) and platelet count (PLT) were recorded for all participants. NLR was calculated simply dividing the Neu value by the Lym value.

The automatic analyzer of the LH 780 Beckman Coulter device (Beckman Coulter Inc.; Brea, CA, USA) was used for blood count analyses in the laboratory of our institution. For laboratory analyses, the original kits of the manufacturer were used. Statistical analysis was conducted using SPSS software (SPSS15.0; SPSS Inc., Chicago, IL, USA). Data were expressed as mean±SD or median (min-max). Independent samples t-test or Mann-Whitney U test were used to compare variables in the study population. A p-value lower than 0.05 was considered statistically significant.

Results

A total of 90 patients with HT and 64 healthy control subjects were enrolled in the study. Mean ages of the HT and control groups were 37±11 and 39±9 years, respectively. The difference was not statistically significant (p=0.27). The HT group consisted of 75 women and 15 men while the control group comprised 56 women and eight men. There was no significant difference between HT and control groups in terms of gender (p=0.47). While thyroid stimulating hormone (TSH), free T4 and free T3 levels of all control subjects were normal, 22 of HT subjects were thyrotoxic (suppressed levels of TSH along with elevated T4 or T3). None of the HT patients were hypothyroid. General characteristics and laboratory data of both study populations are shown in Table 1.

The NLR of HT group (2.1 [1.3-5.8]) was significantly higher than that of the control group (1.9 [0.6-3.3]) (p=0.04).

Discussion

The main finding of our study is that NLR was elevated in HT patients compared with the healthy control subjects. This is the first reported association between HT and NLR.

By 2010, NLR had been introduced as a simple indicator of systemic inflammation in different clinical conditions.[12] Shimada et al. proposed NLR as a reliable predictor of inflammatory burden.[13] C-reactive protein (CRP), which responds immediately to infectious or inflammatory stimulus, is one of the most well-established inflammatory markers and, interestingly, NLR was found to correlate with CRP.[14,15] There are a number of reports studying NLR in various thyroid diseases. Researchers from Taiwan showed that NLR correlated with the size of thyroid tumors.[16] Moreover, elevated NLR was proposed as a negative prognostic factor for survival in subjects with papillary thyroid cancer.[17] Aside from thyroid neoplasm, NLR has also been found to correlate with other types of neoplasms.[18,19] Inflammation plays a critical role in tumor development, progression, clinical presentation and prognosis of cancer.[20] HT is also characterized by a prominent inflammatory burden, which is consistent with lymphocytic inflammation of the thyroid gland;[21] therefore, the increased NLR seen in HT patients compared with controls in this study is likely to be a result of chronic inflammation.

Elevated NLR has been reported in patients with familial Mediterranean fever (FMF) and has emerged as a valuable predictor of the development of amyloidosis.[22] In addition to inflammation, HT is characterized by autoimmunity, which is defined by reactivity to antigens of thyroidal tissue.[23] Both thyroid follicular cells and inflammatory cells, involved in HT, are capable of producing cytokines that may exacerbate the autoimmune process and the inflammatory response;[24,25] therefore, mechanisms similar to those seen in FMF may induce elevated NLR.

TABLE 1 General characteristics and laboratory data of the study population.

		Group		p
		HT group	Control group	
Gender	Men (n)	15	8	0.47
	Women (n)	75	56	
		Mean±SD		
Age (years)		37.3±11.5	39.2±9	0.27
WBC (u/mm^3)		7.5±1.7	7.1±1.5	0.66
Hb (g/dL)		13.6±1.3	13.6±1.4	0.15
Htc (%)		40±3.5	40.8±3.8	0.16
MCV (fL)		86±6	86±5	0.55
PLT (u/mm^3)		299±63	301±63	0.91
TSH (uIU/mL)		1.2±1	1.9±1	0.002
FT4 (ng/dL)		1.5±0.5	1.2±0.2	0.03
FT3 (pg/mL)		4.3±0.9	3.3±0.4	0.03
		Median (min-max)		
Neu (u/mm^3)		4.3 (2.1-9.8)	4.1 (2-6.3)	0.36
Lym (u/mm^3)		2 (0.8-3.5)	2.2 (1.3-4.7)	0.08
NLR		2.1 (1.3-5.8)	1.9 (0.6-3.3)	0.04

HT: Hashimoto's thyroiditis; WBC: white blood cell count; Hb: hemoglobin; Htc: hematocrit; MCV: mean corpuscular volume; PLT: platelet count; TSH: thyroid stimulating hormone; FT4: free thyroxine; FT3: free triiodothyronine; Neu: neutrophil count; Lym: lymphocyte count; NLR: neutrophil-to-lymphocyte ratio.

External administration of thyroid stimulating hormone (TSH) stimulates triiodothyronine (T3) production in lymphocytes.[26] Increased intracellular T3 may stimulate lymphocyte production, causing an elevated lymphocyte count on a CBC. In this study, patients with HT had significantly lower TSH than healthy control subjects. Lower serum TSH might lead to a reduction of intracellular T3 in lymphocytes, which could result in an increase in NLR. On the other hand, HT should be considered as a situation characterized by inflammatory stress, and T3 concentration in lymphocytes has been found to decrease under stressful conditions.[27]

In healthy populations, NLR is increased in the elderly;[28] however, the mean age of the HT patients was not different from the healthy controls in our study so the increase in NLR seen in our study cannot be attributed to this age-related correlation.

Hashimoto's thyroiditis is associated with endothelial dysfunction,[29] which is considered to be an underlying cause of atherosclerosis and may reflect an early stage in the development of atherosclerosis.[30] NLR was suggested to be an independent predictor of cardiac mortality in ischemic heart disease.[31] Moreover, NLR was found to be useful for risk stratification in acute coronary syndrome.[8] A more recent study revealed that NLR was associated with the severity and the prognosis of ischemic heart disease.[32] HT is thought to be associated with endothelial dysfunction, and, thus, with atherosclerosis;[33] therefore, the observation of an elevated NLR in HT, similar to that seen in atherosclerotic diseases, is not surprising.

Although hypothyroidism is more common in HT, initial inflammatory destruction of the gland may cause a transient thyrotoxicosis; therefore, serum T3 and T4 levels were higher in HT patients compared to subjects in present study.

Our study was limited by its retrospective design, which could lead to selection bias, and by the small study cohort that made conclusive interpretation of the results difficult.

Conclusion

NLR is an inexpensive and easily accessible test and, therefore, we suggest that measurement of NLR may be useful in the diagnosis of HT, especially in complicated cases when a diagnosis is uncertain. Larger prospective studies are required to fully assess the usefulness of NLR to diagnose and assess treatment outcomes in HT patients.

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The impact of HSF on endometrium

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Summary

Objective: We conducted the research in order to explore the impact of hydrosalpinx fluid (HSF) on endometrium.
Method: HSF group: 261 patients with HSF scheduled to undergo laparoscopic surgery 3 to 7 days after menstruation in our center. Hysteroscopy would also be performed in order to observe the endometrial morphology during the surgery. Sixty (60) patients would be randomly selected for endometrial biopsy in order to detect the inflammatory cytokines TNF-α and IL-2 mRNA. Non-HSF group: 210 patients with no evidence of HSF due to chronic salpingitis or pelvic adhesion. IVF-ET treatment was performed after eliminating the factor of male infertility and hysteroscopy was conducted before the treatment. Fifty (50) patients underwent endometrial biopsy in order to detect TNF-α and IL-2 mRNA.
Results: Hysteroscopy was performed in 261 patients with HSF and 210 patients without HSF. The incidence rate of endometritis manifestation among these two groups of patients was 37.2% (97/261) and 20.5% (43/210), respectively. The incidence rate of endometritis in the patients with HSF is significantly higher than in the patients without HSF ($p<0.05$). Sixty (60) patients from the HSF group and 50 patients from the non-HSF group were regrouped according to inflammatory and normal manifestation after the endometrial biopsy. There were 49 patients in the inflammatory manifestation group and 61 patients in the normal manifestation group. RT-PCR technology was adopted to detect the expression of inflammatory cytokines TNF-α and IL-2 mRNA in endometrial tissue. The level of TNF-α mRNA expression in endometrial tissues with inflammatory manifestation was higher than in normal endometrium (76.75±11.95 vs. 23.45±9.75, $p<0.01$). There are significant differences between them. The level of IL-2 mRNA expression in endometrial tissues with inflammatory manifestation was higher than that found in normal endometrium (80.56±13.35 vs. 35.12±8.35, $p<0.01$). There are significant differences between them.
Conclusion: Chronic endometritis is related to HSF and may therefore affect endometrial receptivity.

Keywords: HSF, endometrium, immunohistochemistry, polymerase chain reaction.

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Introduction

Endometrial receptivity is an important factor to determine the success of pregnancy and hydrosalpinx fluid (HSF) may have a bad effect on endometrial receptivity through various mechanisms. Using immunohistochemical examination on patients with HSF after endometrial biopsy, Mayer et al.[1] found that endometrial integrin $\alpha_v\beta_3$ of the patients with HSF during implantation window period significantly decreased compared with the non-HSF control group, but the above endometrial integrin had clearly improved in expression after salpinx surgery. As integrin $\alpha_v\beta_3$ is an important index for endometrial receptivity, it can be concluded that HSF influences the endometrial receptivity. In fact, many studies in recent years showed that indexes of endometrial receptivity for HSF patients including endometrial inflammatory re-

sponse, integrin, matrix metalloproteinases, endometrial blood flow, leukemia inhibitory factor are moderately affected.[2-4] Our study explores the connection between HSF patients and chronic endometritis (CE) and verifies whether HSF would influence endometrial receptivity by inducing CE. We observed the general performance of endometrium in patients with HSF, first through hysteroscopy and then collecting endometrial samples from different patients for the detection of TNF-α and IL-2 mRNA in order to assess whether HSF has negative effects on the endometrium.

Method

Study object

Four hundred seventy-one (471) patients undergoing infertility treatment in the Reproductive Medicine Centre due to salpingitis from July 2010 to June 2014 were chosen. Their average age was 31.5 years and infertility lasted from 2 to 9 years.

Inclusion criteria

- (1) The female partner of infertile couples needing treatment due to salpingitis.
- (2) Age range from 25 to 35 years old.
- (3) Normal menstrual cycle lasting from 25 to 35 days with a variation of 3 days.
- (4) Weight range from 45 to 70 kg and body mass index between 18 and 25 kg/m^2.

Exclusion criteria

- (1) Infertility due to unknown reasons other than salpingitis.
- (2) Routine examination of male semen abnormal more than twice.
- (3) Female partner with one of the following endocrine abnormalities: polycystic ovary or polycystic ovary syndrome, hyperprolactinemia.
- (4) Female partner presenting abnormal cervical cytological examination: HPV infection or atypical cell hyperplasia or more severe lesions indicated by TCT.
- (5) Female partner's baseline endocrine levels (menstruation D2 ~ D5) FSH and/or LH > 10 IU/mL.
- (6) Acute inflammation of the genital tract.
- (7) Organic lesions present including submucosal uterine fibroids, endometrial polyps, uterine adhesions.

Diagnostic criteria

HSF group: HSF was indicated by hysterosalpingography (HSG). Non-HSF group: no HSF found after HSG that would be caused by chronic tubal inflammation or pelvic adhesion.

Experiment group

HSF group: 261 patients with HSF scheduled to undergo laparoscopic surgery 3 to 7 days after menstruation in our center. Hysteroscopy would also be performed in order to observe the endometrial morphology during the surgery. Sixty (60) patients would be randomly selected for endometrial biopsy in order to detect the inflammatory cytokines TNF-α and IL-2 mRNA.

Non-HSF group: 210 patients with no evidence of HSF due to chronic salpingitis or pelvic adhesion. IVF-ET treatment was performed after eliminating the factor of male infertility and hysteroscopy was conducted before the treatment. Fifty (50) patients underwent endometrial biopsy in order to detect TNF-α and IL-2 mRNA.

Experiment methods

Methods and procedures for endometrial examination with hysteroscopy

- (1) Evaluation time: the best period is the early to the middle stage of endometrial proliferation, i.e., 3 to 7 days after menstruation.
- (2) Anesthesia: Hysteroscopy can be performed in outpatient setting without anesthesia and few patients need intravenous or general anesthesia. In the case of inpatients who prepare to undergo combined laparoscopic surgery, general anesthesia was used.
- (3) The objective lens of hysteroscope is placed into the cervical canal slowly under direct vision after routine disinfection and speculum placement. At the same time, physiological saline is applied for cervical canal expansion and uterine distention, the distending pressure is 100-120 mmHg.
- (4) Endometrial morphology observation: Normal uterine cavity was covered by newly formed smooth endometrium, yellowish and reddish, with few blood capillary and open glandular ducts (Figure 1). Endometritis patients often show endometrial hyperemia, edema and exudation, even necrosis. In this study, the biopsy would be performed only for patients with normal endometrial appearance and inflammatory manifestations like endometrial hyperemia, edema etc. Other lesions such as endometrial polyp, submucosal myoma of uterus etc. were excluded from our study. Diagnostic criteria for CE under hysteroscope can refer to the description of Zolghadri et al.:[5] endometrial hyperemia presents a crimson red or fire red color and the subepithelial vascular network is significantly dense and thickened (Figure 2).
- (5) Sampling: endometrial tissue was taken as specimen by a slim catheter (Wallace) after hysteroscopy. The sam-

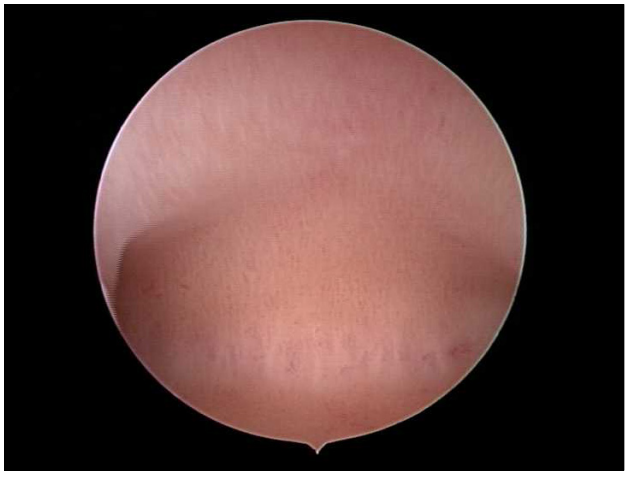

FIGURE 1 Normal uterine cavity: smooth endometrium, in pale red color without abnormal blood vessel.

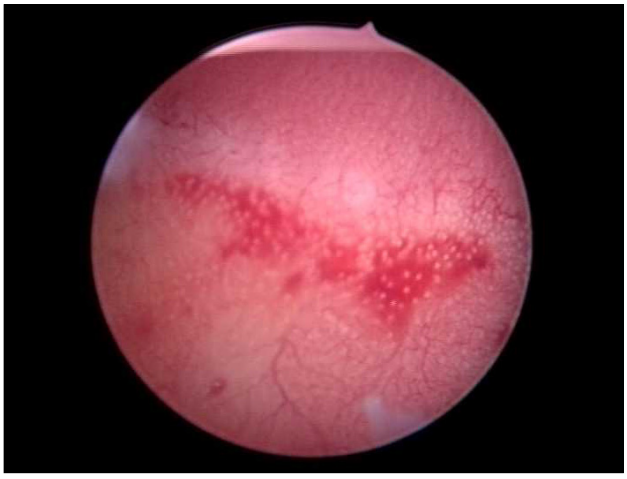

FIGURE 2 Uterine cavity with inflammatory manifestation: endometrium presents purple red or red colors after hyperemia and the subepithelial vascular network is significantly dense and thickened.

ple was then rinsed by physiological saline in order to reduce blood contamination as much as possible, inserted into a 1.5 mL Ep tube with high pressure sterilization and DEPC water treatment and then stored in a freezer at -70°C ultra-low temperature in order to extract RNA from the tissue during RT-PCR detection.

Detection of the expression of TNF-α and IL-2 mRNA in endometrium with RT-PCR

Relevant reagents: Total RNA extraction kit with Trizol reagent produced by Invitrogen Company (USA). RNA reverse transcription kit, 10 mmol/L dNTPs, 5 U/ mu lTaq DNA polymerase and agarose were all products of Promega Company. Synthesis of PCR primers was entrusted to Sangon Biotech (Shanghai) Co., Ltd. and TNF-α primer sequences were: upstream primer 5-G A G T G A C A A G C C T G T A G C C C-3′, downstream primer 5'-G C A A T G A T C C C A A A G T A G A C C-3' with the amplified product length of 363 bp (Figure 3). And the primer sequences of Internal reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were: upstream primer 5′-C C A C C A C C A T C T T C C A G G A G-3′, downstream primer 5′-C C T G C T T C A C C A C C T T C T T G -3′ with the amplified product length of 572 bp. IL-2 primer sequences were: upstream 5′-C T G G A G C A T T T A C T G C T G G A T-3′, downstream 5′-G C C T T C T T G G G C A T G T A A A A C-3′ with the amplified product length of 110 bp (Figure 4).

The specific procedure in the RT-PCR experiment was as follows:

- (1) Treatment of endometrium: about 100 mg fresh frozen endometrial tissue sample was cut into small pieces, cleaned with D-Hanks liquid and centrifuged for 12 minutes in a low temperature centrifuge at a temperature of 4°C and 10,000 rpm. The supernatant was removed and the tissue sediments were washed for 2 to 3 times with D-Hanks liquid.
- (2) Total RNA extraction: total RNA was extracted according to procedures in the manual of total RNA extraction kit with Trizol reagent. The optical density values of A_{260} and A_{280} were determined using a DU800 spectrophotometer from Beckman Coulter with a ratio of ranging from 1.8 to 2.0. The concentration of total RNA was then calculated. At the same time, total RNA electrophoresis yielded three strips of 28S, 18S and 5S or two strips of 28S and 18S, showing that RNA quality met the requirements (Figure 5).
- (3) cDNA synthesis: reverse transcription kit from Invitrogen Company was adopted to synthesize cDNA according to the manual. The cDNA obtained from reverse transcription was used as a template for PCR reaction.
- (4) PCR reaction: cDNA of 2 μL, primer of 200 nmol/L, dNTP of 150 μmol/L, Taq DNA polymerase 1 U and corresponding buffer solution were included in the 25 μL reaction system. TNF-α, IL-2 and internal reference GAPDH in type 9600 DNA cycler (Perkin Elmer Cetus) were amplified according to the reaction conditions. The amplification conditions of the three genes were as follows: pre-denaturation for 3 minutes at 94°C in the beginning; repeated cycling 35 times for 30 seconds at 94°C, for 30 seconds at 55°C, for 1 minute at 72°C; 7 additional minutes at 72°C in the end.

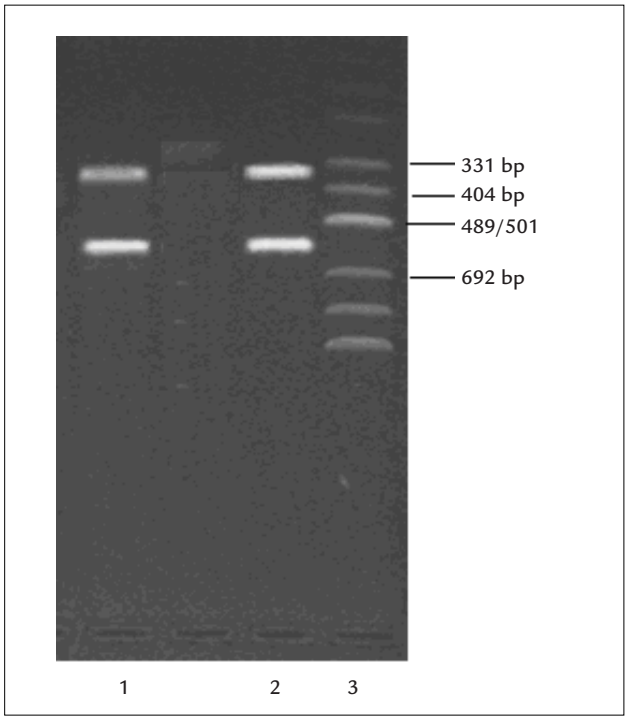

FIGURE 3 Electrophoresis track: 1. TNF-α/GAPDH of endometrium with normal manifestation. 2. TNF-α/GAPDH of endometrium with inflammatory manifestation. 3. pUC Mix Marker.

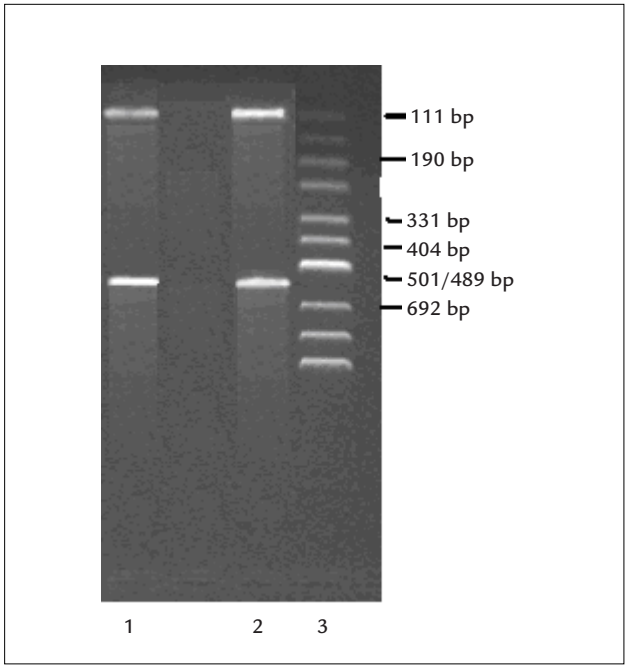

FIGURE 4 Electrophoresis track: 1. IL-2/GAPDH of endometrium with normal manifestation. 2. IL-2/GAPDH of endometrium with inflammatory manifestation. 3. pUC Mix Marker.

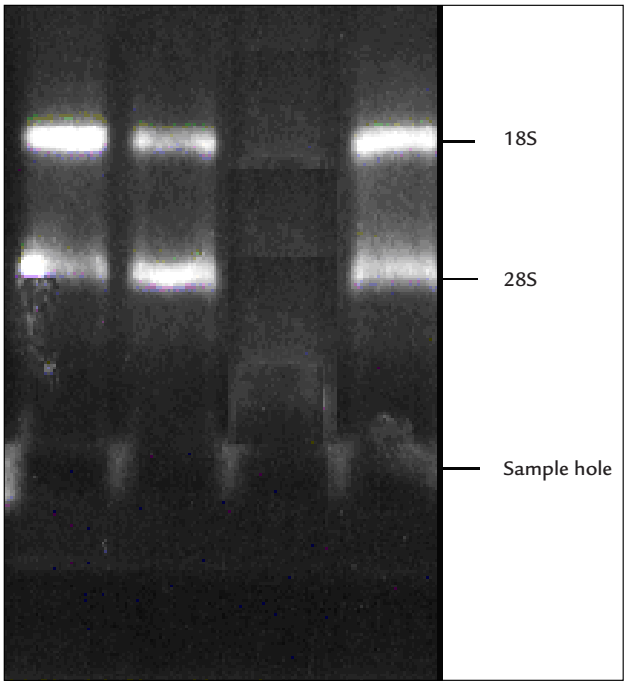

FIGURE 5 Result of total RNA electrophoresis extracted from endometrial tissues.

- (5) Identification of amplification products: Absorbance scanning for TNF-α, IL-2 and GAPDH was performed by using GD 2000 gel scanning and analyzing system D with pUC Mix treated as molecular weight standard after taking PCR amplification product of 10 μL, 2% of agarose gel electrophoresis and ethidium bromide staining (including the ethidium bromide with a final concentration of 0.5 μg/mL). And then the ratio of gene expression of TNF-α and IL-2 to that of GAPDH should be calculated respectively.

Statistical method

SPSS 11.0 statistical software was used for data analysis. The obtained data was expressed as $\overline{x} \pm s$ and tested with t-test. And enumeration data was detected by χ^2 check. Statistical significance was defined as $p<0.05$.

Results

Hysteroscopy result

In this study, hysteroscopy was performed in 261 patients with HSF and 210 patients without HSF. The incidence rate of endometritis in these two groups of patients were 37.2% (97/261) and 20.5% (43/210), respectively. As shown in Table 1, the incidence rate of endometritis in the patients with HSF is significantly higher than that of patients without HSF.

TABLE 1 The ratio of endometritis in three groups of patients evaluated by hysteroscopy.

Group	Total number of cases (n)	Number of cases with inflammatory manifestation (n)	Inflammatory manifestation rate (%)
HSF group	261	97	37.2 (97/261)
Non-HSF group	210	43	20.5 (43/210)*

p<0.05 * compared with HSF group.

Sixty (60) patients from HSF group and 50 patients from non-HSF group were regrouped according to the inflammatory or normal status after endometrial biopsy. There were 49 patients in the inflammatory manifestation group and 61 patients in normal group. RT-PCR technology was adopted to detect the expression of inflammatory cytokines TNF-α and IL-2 mRNA in endometrial tissue.

Result of RT-PCR detection of TNF-α and IL-2 mRNA in endometrium

- (1) The level of TNF-α mRNA expression in endometrial tissue with inflammatory manifestation was higher than that seen in normal endometrium (p<0.01). There was significant differences between them (Table 2).

TABLE 2 RT-PCR detection of the levels of TNF-α expression in two groups of subjects investigated.

Group	Number of cases	Relative content of TNF-α (%)
Inflammatory manifestation group	49	76.75±11.95
Normal manifestation group	61	23.45±9.75

p<0.01

- (2) The level of IL-2 mRNA expression in endometrial tissues with inflammatory manifestation was higher than in normal endometrium (p<0.01). There was significant difference between them (Table 3).

TABLE 3 RT-PCR detection of the levels of IL-2 expression in two groups of subjects investigated.

Group	Number of cases	Relative content of IL-2 (%)
Inflammatory manifestation group	49	80.56±13.35
Normal manifestation group	61	35.12±8.35

p<0.01

Discussion

Embryo quality and endometrial receptivity are two key factors that influence the clinical outcome of pregnancy. The embryo implantation process undergoes many special changes, involving a series of signal transduction processes both cellular and molecular. Hormones produced by the ovaries can initiate autocrine or paracrine activities of various downstream cytokines, trigger expression of integrin and other adhesion molecules and then mediate the mutual recognition between embryo and endometrium in order to prepare for implantation.[6] For example, estrogen and progesterone can regulate the expression of HOXA10 in endometrium, which affects the expression of integrin β3 in endometrium, being closely related to the change seen in endometrial hyperplasia and pregnancy.[7] Almost all of the studies for endometrial receptivity show that HSF hinders embryo implantation by reducing endometrial receptivity.[1-3] Since HSF is a manifestation of sequelae of pelvic inflammatory disease, it is speculated that HSF may be related to CE. However, the related research is still rarely reported up to now. The value of hysteroscopy in evaluating intrauterine environment has already reached a consensus that the correct treatment of lesions after hysteroscopy does not only improve the pregnancy rate, but also has a predictive value on the pregnancy outcome of IVF treatment after hysteroscopy.[8] But, in hysteroscopy, people usually pay close attention to the treatment of endometrial polyps, endometrial hyperplasia, submucosal uterine myoma, uterine adhesion and other uterine organic lesions, and often ignore the adverse effects of minor lesions (like CE) in intrauterine environment. CE may lead to infertility and abortion. It mainly presents as endometrial matrical edema, local punctate or diffuse hyperemia,[5,9] under hysteroscope. Under a microscope, in turn, delayed maturation of endometrium and stroma (influenced by inflammation) can be seen, while capillary and venous sinus have enlarged and remained in that state, with presence of many different plasma cells and lymphocytes infiltrated in the endometrial stroma. The presence of plasma cells is one of the mainstays of CE diagnosis. Recently, a retrospective analysis reported the sensitivity and specificity of hysteroscopy in the diagnosis of CE compared with a pathological analysis: the incidence rate of the abnormities such as endometrial polyp, hyperemia etc. is 66.3% with hysteroscope, while the incidence rate of CE reported after pathological analysis is 43.6%. Taking pathological diagnosis as standard for calculation, the sensitivity and specificity of hysteroscopy in the diagnosis of CE is 35.23% and 67.54%, respectively.[10] It has been found that, compared with histological examination, hysteroscopy can reflect more effectively inflammatory conditions of the uterine cav-

ity but better ways to diagnose CE are still needed. Whether CE affects embryo implantation is still controversial; the high incidence rate of CE in patients with embryo implantation failure is an indisputable fact.[10,11]

Inflammatory cytokines are important mediators for the occurrence of CE and both TNF-α and IL-2 play an important role in the process of inflammatory response.[12] TNF-α is mainly expressed by mononuclear macrophages, CD4+Th1 cells and natural killer (NK) cells etc. in the immune system. Besides the expression in immune cells, TNF-α can also be expressed in reproductive tissues such as ovary, salpinx, uterus, placenta etc. And it also takes part in the process of gametogenesis, embryonic development, follicle growth and steroid hormone synthesis etc., through autocrine and paracrine action. There must be a right amount of TNF-α in pregnant women to maintain pregnancy, but TNF-α at high concentrations may lead to a series of inflammatory lesions, stimulate the production of multiple inflammatory factors such as IL-1, IL-6, NO etc. and result in the occurrence of inflammation and damage in tissues by activating inflammatory cells and upregulating adhesion molecules, NO and oxygen free radicals. After detecting the levels of IL-6 (interleukin-6), IL-1β and TNF-α in menstrual blood of the patients diagnosed as CE by hysteroscopy and histology in follicular phase of the previous menstrual cycle, Tortorella et al.[13] believed that proinflammatory cytokines IL-6 and TNF-α can be used as a biomarker for CE. TNF-α also has a direct toxic effect on endometrium and harms the decidua vessel, which brings about contraction of vascular smooth muscle, embolism of the embryonic blood-supply system and tissue necrosis.[14] IL-2 is the glycoprotein produced and secreted by activated T cells with a molecular weight of about 15kD. At the same time, it is necessary for T cell proliferation, as well as a key mediator of the immune response. It plays a key role in the cellular immune response and possesses a mechanism of promoting the biological activity of T cells and B cells.[15] IL-2 may also be expressed by endometrial glandular cells but excessive expression of IL-2 may affect embryo implantation.[16] IL-2 level reflects the body's cellular immune state to some degree and IL-2 has a function of anti-tumor, anti-microbial infection, also inducing graft rejection, autoimmunity and immune regulation. Therefore, detection of IL-2 levels is a sensitive index to assess the immune activation status of the body. TNF-α and IL-2 are all cytokines secreted by Thl cells and play a key role in the inflammatory response. Piccinni et al.[17] found that Th1 cytokines can downregulate the expression of LIF, and IL-2 is an indispensable cytokine in embryo implantation. HSF, in turn, may influence the endometrial receptivity by decreasing the expression of LIF in the endometrium during the implantation window period.[16] After hysteroscopy, we found that the incidence rate of the inflammatory manifestation of endometria in patients with HSF and the expression of TNF-α and IL-2 in endometrial issues with inflammatory manifestation increased significantly, indicating that inflammatory cytokines such as TNF-α and IL-2 in the endometrium of patients with HSF were involved in the inflammatory manifestation of endometrium. In the process of embryo implantation, the matrix was in a state of immune tolerance with Th2 type immune response in a dominant position. The high expression of TNF-α and IL-2 in endometrium of patients with HSF may also indicate that the local immune balance of the endometrium biases towards the Th1 type immune response, which affects embryo implantation.

Due to limitation of experimental conditions, we only tested the typical TNF-α and IL-2 instead of detecting a large number of inflammatory cytokines. Detection of endometrial receptivity only involves inflammatory response, which may result in probable error for the conclusion. Further studies on the impact of HSF on endometrial receptivity are still needed.

Conclusion

CE is related to HSF, and endometrial receptivity may be influenced by HSF.

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Implementation of a standardized out-of-hospital management method for Parkinson dysphagia

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Summary

Objective: Our objective is to explore the effectiveness and feasibility of establishing a swallowing management clinic to implement out-of-hospital management for Parkinson disease (PD) patients with dysphagia.
Method: Two-hundred seventeen (217) voluntary PD patients with dysphagia in a PD outpatient clinic were divided into a control group with 100 people, and an experimental group with 117 people. The control group was given dysphagia rehabilitation guidance. The experimental group was presented with the standardized out-of-hospital management method as overall management and information and education materials. Rehabilitation efficiency and incidence rate of dysphagia, as well as relevant complications of both groups were compared after a 6-month intervention.
Results: Rehabilitation efficiency and the incidence rate of dysphagia including relevant complications of patients treated with the standardized out-of-hospital management were compared with those seen in the control group. The differences have distinct statistics meaning ($p<0.01$).
Conclusion: Establishing a swallowing management protocol for outpatient setting can effectively help the recovery of the function of swallowing, reduce the incidence rate of dysphagia complications and improve the quality of life in patients with PD.

Keywords: Parkinson disease, swallowing disorders, ambulatory care.

Introduction

Parkinson disease (PD) is a degenerative disease of the nervous system. Lesions always involve the nerves and muscle of swallowing organs such as mouth, face, throat and esophagus, causing dysphagia. Dysphagia can lead to complications such as malnutrition, mis-inhalation, aspiration pneumonia, and is one of the main reasons for death, disability[1,2] and repeated hospitalization among PD patients. According to the literature, 35 to 80% of PD patients have dysphagia.[3]

To date, the pathogenesis of PD is not clear. At present, drug treatment and deep brain stimulation therapy are effective for improving PD patients' limb functions, but improvement of swallowing function is not clear and to date remains controversial.[4-8] In recent years, studies have shown that swallowing therapy machine has some effect on vascular Parkinson syndrome with dysphagia. But it still needs to be further verified.[4-9] Gadenz et al.[10] applied transcranial magnetic stimulation to one patient with PD and dysphagia and one patient with Alzheimer disease and dysphagia. There was an effect on both of them.[10] But this therapy currently needs large-scale clinic tests. At present, many studies have shown that swallowing training may help coordinate PD patients' swallowing movement, and reduce the risks caused by dysphagia and improve the patients' life quality.[11,12]

Now, the world-recognized treatment method for Parkinson combines medical intervention and rehabilitation along with a concept of long-term comprehensive attention to the patient.[13] At present, remedial method is the main treatment for Parkinson in China. Rehabilitation is only carried out in some developed cities, with a few reports focusing on Parkinson dysphagia in hospitals alone.[14]

In February 2015, our department established a swallowing management clinic aimed at the features of PD patients with dysphagia and the existing difficulties in current therapy. Nurses specifically trained to improve the patients' swallowing function and PD outpatient doctors participated as a team to comprehensively manage and apply the standardized out-of-hospital management method for PD patients with dysphagia. The effect is remarkable. The report is as follows.

Method

Object

PD patients with dysphagia who sought the clinic were voluntarily divided into a control group and an experimental group. The control group had 100 people with an average age of 69.3±11.3 years; 59% were male. The experimental group had 117 people with an average age of 71.4±12.7 years, 60.6% being male. All of them met the diagnostic criteria for PD established by the UK Brain Bank. Dementia was ruled out through the mini-mental filter. Patients whose swallowing function is below level 3 were referred for hospitalization. PD patients presenting level 6-3 dysphagia were divided in two groups and displayed no distinct difference (p>0.05) (Table 1). Patients with background comorbidities such as high blood pressure, diabetes, coronary heart disease, surgical history and disease course showed no distinct difference (p>0.05) (Table 2).

TABLE 1 Comparison of dysphagia between the control group and experimental group.

Groups	Control group	Experimental group
Level 6	11	15
Level 5	23	29
Level 4	53	58
Level 3	13	12

TABLE 2 Comparison of patient characteristics between the control group and experimental group.

Groups	Control group	Experimental group
Average age	69.3±11.3	71.4±12.7
Male proportion	59 (59%)	71 (60.6%)
Disease course	5 ±3.8	5±3.9
Coronary heart disease	11 (11%)	18 (15%)
Diabetes	1 (1%)	0
High blood pressure	5 (5%)	9 (7.6%)
Surgical history	3 (3%)	4 (3.4%)

Standardized out-of-hospital management intervention method

Long-term attention and overall management

- The establishment of a swallowing management clinic: Aimed to serve patients who have dysphagia caused by chronic or other diseases who cannot recover with short-term treatment inside the hospital, and need long-term rehabilitation and intervention outside the hospital.
- The establishment of swallowing archives: The clinic's nurses screen swallowing function of every PD patient who has seen a doctor at the clinic, and establish swallowing archives which include the patients' basic information/characteristics, specific diagnosis, relevant eating complaints, the results of swallowing function screening, dysphagia intervention strategy, health history, personal history, family history, contact information, reexamination time and method implementation courses, etc.
- Patients with swallowing problems should receive long-term attention and periodic re-examination. The different intervention strategies are applied according to the swallowing problems at different stages to make sure patients eat safely and receive enough nutrition.

Multimedia training combined with feedback method to raise awareness and educate

- The courses are designed by the swallowing team, they are easy to understand and carry out. The specialist provides theoretical training using a PPT. The contents are mainly about dangers caused by dysphagia and eating considerations. The skill training mainly presents oral muscle exercises, tongue muscle training exercises, pronunciation training, effective cough training, eating training and compensatory training with video and presentation.
- Both the patients and their families are subject to the course. The course lasts 30 m.
- After the training, a feedback step takes place to know how much information the patients and their families have mastered.

The training contents of out-of-hospital rehabilitation

According to their dysphagia screening results and function evaluation situation, grading and performance of dysphagia is determined and the targeted rehabilitation training is given.

Basic swallowing skill training is for patients with dysphagia in oral phasis as follows.

- Oral muscle exercises: lips shrinkage, tightening lips, pursing lips, bared teeth, maximizing mouth open,

closing mouth, taping teeth, cheek blowing and gargling training. Each exercise should be done 10 times every session, twice a day.

- Tongue muscle exercises are for patients who can automatically exercise their tongue and lead it to do tongue extension movement. Front, back, up, down, left, right: the movement in every direction lasts for at least 5 seconds. Each exercise should be done 10 times at every session, twice a day. Patients who cannot automatically move their tongue can have the aid of tongue muscle rehabilitation machine to do passive training. Each movement can be done 10 times at every session, once a day.

Mis-inhalation protection training is for patients with low volume, weak cough power and poor airway protection ability.

- Pronunciation training: vowels pronunciation training like (i:) (u:) (a:) (e). It should be gradually stretched at every session daily, in the mornings and at night. Patients are encouraged to sing at least one song every day.
- Effective cough training: patients breathe with nostrils, then hold breath for 3 to 5 seconds and try deeply to cough to effectively clean up airway.
- Pharynx cold stimulation and empty swallowing training: patients with dysphagia in pharyngeal phase and without any heart diseases can use pharynx cold stimulation and empty swallowing training twice a day.

Eating training and guidance outside the hospital

The eating prescription will be given based on an eating evaluation.

The eating posture should be the natural sitting position. Those who are still weak can use semi-sitting posture, making sure that the head is above 30 degree. Eating amount per morsel: width of the open mouth, chewing ability and control of food in the mouth, triggering of swallowing reflection and the amount of food remaining in the mouth after swallowing are all aspects observed during the eating evaluation. Food property selection: we should choose foods that have uniform density, are compact and distort easily when passing the esophagus, without leaving any residues on the mucosal.

Patients should avoid eating dry, hard, loose, and graininess food. The eating environment should be quiet, talking and laughing being avoided. Patient attention should be kept. Compensatory strategies:

- Swallowing with head up is mainly used for dysphagia patients in oral phase. Gravity should be used to send foods from mouth to pharynx.
- Swallowing with head down is mainly used for patients with poor airway protection ability.
- Swallowing with head nodding is the combination of swallowing with head up and down. It is also useful to reduce the residues on epiglottis and piriform recess to prevent mis-inhalation.

Inspect implementation by WeChat platform

- The writer formed a PD management WeChat group and invited the experimental group and their families to participate. Three specially trained nurses will be in the group.
- Relevant health education knowledge on dysphagia is periodically published. The relevant complications and dangers of dysphagia are published on Monday, and considerations and knowledge on dysphagia with a video about rehabilitation and eating skills are released on Friday. The release time is from 8:00 to 10:00 in the morning. It is never sent at nap time or at night to avoid disturbing the patients and their families.
- Patients can send their questions about swallowing and nutrition at any time, which will be answered timely.
- We focus on every patient while implementing the rehabilitation training, offering them supervision and guidelines, ensuring the efficiency of measure implementation, and reminding them to undergo periodic re-examination. The intervention strategy is given according to different re-examination situations.
- We record the patients' feedbacks, as well as their problems, and immediately contact the patients who do not undergo re-examination to know their situations and provide them with targeted information and education materials to make them adhere to the rehabilitation method and undergo periodic re-examination.

Intervention method for the control group

The control group will be given face and tongue training and eating considerations pursuant to the regular guidance method. They will not be given out-of-hospital management intervention.

Statistic analysis

The dysphagia rehabilitation efficiency and mis-inhalation incidence rate will be compared between the control group and the standardized out-of-hospital management group after 6 months by using SPSS17.9 statistic

software to analyze statistics. The $p<0.01$ difference is meaningful for statistics. Rehabilitation efficiency means the rate of patients' function recovered and improved after using intervention method.

Results

Compared with the control group, patients of dysphagia in the experimental group have apparently higher recovery efficiency through the method of standardized out-of-hospital management. The control group's recovery efficiency is 17%, while the experimental group's is 68.3%, and mis-inhalation rate is 5.1% in the experiment group, while this rate is 22% in the control group. ($F=21.9$, $p<0.01$) (Tables 3 and 4) (Charts 1 and 2).

TABLE 3 Comparison of PD dysphagia recovery efficiency between the control group and the experimental group.

Group	Control group		Experimental group	
	Cases	Proportion	Cases	Proportion
Level 6	5	45%	13	86.7%
Level 5	7	30.4%	23	78.2%
Level 4	6	11.3%	38	65.5%
Level 3	1	7.7%	6	40%

TABLE 4 Comparison of PD dysphagia mis-inhalation rates between the control group and the experimental group.

Group	Control group		Experimental group	
	Cases	Proportion	Cases	Proportion
Level 6	-	-	-	-
Level 5	1	4.3%	-	-
Level 4	13	24.5%	4	6.8%
Level 3	8	61.5%	2	13.3%

Discussion

The results show that patients who have low level of dysphagia have higher recovery efficiency.

Oral stage is most apparent in early PD dysphagia, and the training of basic skills will be more targeted. Therefore, the building of swallowing management clinics, screening of early dysphagia for PD patients and early out-of-hospital training can maintain the patients' normal swallowing and postpone the progress of dysphagia.

PD dysphagia intervention can be continuously implemented through long-term care and integrated management method, which helps deal with the swallowing problems of different disease stages timely.

PD is a chronic and progressive disease, and PD patients are mainly treated with out-of-hospital health treatments and medicine taking in clinics, and with disease progression, patients will develop the symptom of dysphagia in swallowing and esophagus stages, and therefore, the intervention should make the corresponding adjustment.[15] The studies by Luchesi KF also show that the swallowing ability can be improved through compensation and rehabilitation, which needs a long-term management process.[16] Implementing long-term full concentration methods, making patients check their illness periodically, finding problems timely and adjusting intervention timely can make the intervention more targeted and efficient, ensuring the feeding safety of PD patients.

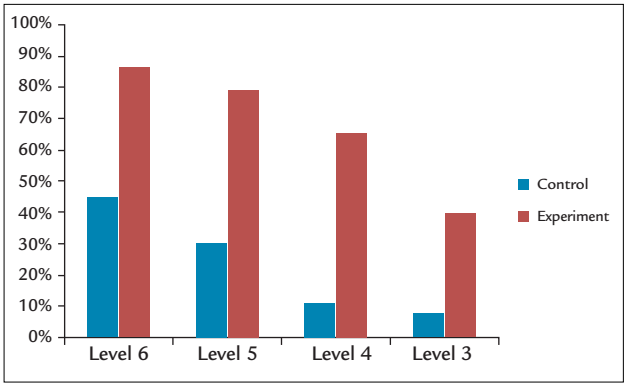

CHART 1 Comparison of PD dysphagia recovery efficiency between the control group and the experimental group.

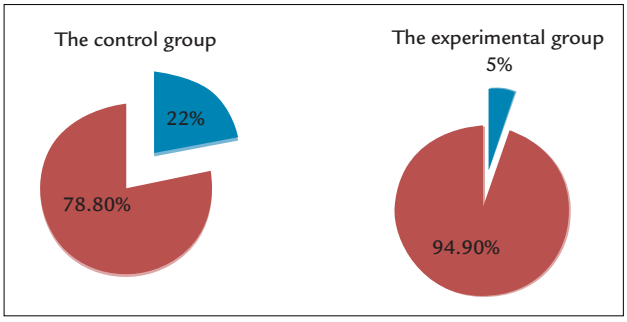

CHART 2 Contrast of PD dysphagia mis-inhalation rates between the control group and the experimental group.

Multimedia promotion and education with feedback method can improve the accuracy of dysphagia out-of-hospital recovery training, increase recovery rate and reduce the risk of dysphagia as a complication.

It is easier to understand and master the health information through the multimedia. The feedback method, used to evaluate the patients' understanding and mastery of the information provided by doctors, is a type of teaching strategy that can minimize the risk of misunderstanding the information provided.[17,18] Applied in

propaganda and education, the feedback method can improve patients' self-management ability and lower their hospital admission rate.[19,20] PD patients often easily forget and have limited ability to understand the information because of their decline in memory or logical thinking, which will directly impact the effectiveness of promotion and education. Therefore, combining images and onsite presentation with a feedback method for specific instructions can ensure out-of-hospital recovering training accuracy.

The out-of-hospital swallowing management method applied in swallowing management clinics can accomplish a win-win situation for patients and doctors and achieve multiple purposes.

Usually, the nursing staff participates in the tasks of screening and instructing, but rarely takes into consideration the objective assessment of the patients' organic functions and specific recovery treatment strategy.

The WeChat platform can promote the out-of-hospital dysphagia recovery training compliance of PD patients to ensure the effect of recovery training.

The WeChat platform, which supports the sending of text, images, voice and video, is an instant messenger introduced by Tencent Inc, with the characters of quick recovery, free spending, multiple platforms crossing and instant messaging. With the popularity of smart phones, WeChat has become an important platform for people's daily life, work and entertainment.[21,22] PD patients can view and learn at any time through the WeChat. Through the platform, nurses can follow the patients' recovery training at any time and instruct and supervise and urge them in time, thus promoting the patients' self-management efficiency, strengthening and inspiring patients' confidence in overcoming their illnesses and ensuring recovery training efficiency.

Conclusion

With the aging of the Chinese society, PD has become the third largest chronic nervous disease affecting the quality of life of older individuals in China, with an annual morbidity rate of 1-20%.[23,24] At present, there are 4 million PD patients in the world, half of which in China.[25] Dysphagia is bad and dangerous for PD patients' daily life and health. Provided that the patients are treated in hospital, it not only increases their financial burden, but also is not convenient for long-term recovery treatment, which cannot ensure safety while feeding of patients with chronic and progressive disease combined with dysphagia. Therefore, it is definitely necessary to build the swallowing management clinic for implementing standardized out-of-hospital management to promote or maintain PD patients' swallowing function and lower the rate of complications. Moreover, the experiment shows that swallowing management clinic for implementing standardized out-of-hospital management has great achievement in dealing with PD dysphagia, so it is feasible.

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Oncological results of surgical treatment versus organ-function preservation in larynx and hypopharynx cancer

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Summary

Introduction: Since the beginning of the 1990s, non-surgical radiochemotherapy treatment has become popular with the prospect of maintaining oncological results and preserving the organ in patients with advanced squamous cell carcinoma of the larynx and hypopharynx. However, subsequent studies demonstrated increased recurrence and mortality after the non-surgical treatment became popular.
Objective: To compare the oncological results of surgical and non-surgical treatments of patients with larynx and hypopharynx cancer and to evaluate the variables associated with disease recurrence.
Method: This is a retrospective cohort study of 134 patients undergoing surgical (total or partial laryngectomy) or non-surgical (isolated radiotherapy, chemotherapy or induction chemotherapy followed by radiotherapy and chemotherapy) treatment, with 62 patients in the surgical group and 72 in the non-surgical group.
Results: Disease-free survival rates were higher in the surgical group (81.7% vs. 62.2%; p=0.028), especially in III/IV stages (p=0.018), locally advanced tumors T3 and T4a (p=0.021) and N0/N1 cases (p=0.005). The presence of cervical lymph nodes, especially N2/N3, was considered a risk factor for disease recurrence in both groups (HR=11.82; 95CI 3.42-40.88; p<0.0001). Patients not undergoing surgical treatment were 3.8 times more likely to develop recurrence (HR=3.76; 95CI 1.27-11.14; p=0.039).
Conclusion: Patients with larynx or hypopharynx cancer non-surgically treated had a poorer disease-free survival, especially in cases with locally advanced tumors (T3 and T4a) and in which the neck was only slightly affected (N0/N1).

Keywords: laryngeal neoplasms, hypopharyngeal neoplasms, carcinoma, squamous cell, laryngectomy, radiotherapy, drug therapy.

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Introduction

Traditionally, the treatment of squamous cell carcinoma of the larynx and hypopharynx has been performed by means of surgical resection, followed or not by adjuvant treatment, with total laryngectomy plus neck dissection being the procedure of choice in most cases. Despite its good oncological result, total laryngectomy is a treatment option that involves great impact on the patient's quality of life, mainly due to the presence of definitive tracheostoma and loss of laryngeal voice.[1]

Since the beginning of the 1990s, some studies on radiotherapy-based treatment[2] and its association with chemotherapy[3] have shown oncological results similar to those obtained with surgical treatment, with some probability of preserving the larynx. Initially, sequential chemotherapy and radiotherapy were proposed. Subsequently, the concurrent approach yielded superior results.[4,5] Organ-preserving protocols based on radiochemotherapy, including new induction chemotherapy protocols,[6] have become popular, whereas the open surgical approach has

become an exception, often reserved for rescue situations when primary treatment has failed.[7]

In the United States, however, subsequent studies have shown a decrease in overall survival among those patients, as opposed to improved results with the treatment of other tumors, coinciding with the indication for non-surgical treatment having become more popular.[8,9] In addition, anatomically preserving an organ does not necessarily mean functionally preserving it.[10] Many patients undergoing non-surgical treatment show great difficulty in reassuming the laryngeal functions of phonation and airway protection.

Currently, it is accepted that, in patients with locally advanced tumors (T4a), the best oncologic result is obtained with surgical treatment, which, however, often involves complete organ resection. Nevertheless, in case of moderately advanced tumors eligible for total laryngectomy (T3 and T4a, selected), there still is controversy regarding the oncological results of organ-preserving protocols.[11]

Thus, our study's objective was to compare the oncological results of surgical and non-surgical treatments of patients with larynx and hypopharynx cancer and to evaluate the variables associated with disease recurrence.

Method

This is a retrospective cohort study, approved by the Institutional Ethics Committee, protocol number 228/14, including patients undergoing surgical treatment (total or partial laryngectomy) and non-surgical treatment (isolated radiotherapy, radiotherapy concurrently with chemotherapy, or induction chemotherapy followed by radiotherapy and chemotherapy) for squamous cell carcinoma of the larynx or hypopharynx, in a tertiary referral oncological hospital oncology from 2009 to 2013.

Inclusion criteria were patients with neoplasia with histopathological confirmation of squamous cell carcinoma of the larynx or hypopharynx. Patients with distant metastasis diagnosed prior to initiation of treatment, patients considered inoperable, compromised margins when undergoing surgical treatment, cases undergoing treatment without curative purposes (palliative chemotherapy or hypofractionated radiotherapy) and patients without adequate follow-up were excluded. Thus, the sample was comprised of 134 patients, of whom 62 were in the surgical group (followed or not by adjuvant radiotherapy and/or chemotherapy) and 72 in the non-surgical group, receiving different therapeutic modalities. The standardized radiotherapy dose used was 5,040 cGy on the surgical bed of the patients operated on, 7,000 cGy to the primary tumor and 6,400 cGy for the lymphatic drainage areas, fractionated in doses of 200 cGy each, for the patients in the non-surgical group.

Statistical analysis

We describe the values we obtained from studying each quantitative variable as means and standard deviations, whereas we use absolute and relative frequencies for the qualitative variables. The distributions were defined as parametric by the Kolmogorov-Smirnov test. We used the Student's t-test to compare the means and the Chi-square test to compare the frequencies between groups of qualitative variables. The Kaplan-Meier method was used in survival analyses. The Log-rank test was used in the comparison between the curves and the Cox regression model for calculating the hazard ratio (HR) with the respective 95% confidence interval (95CI) in the multivariate analysis. In all of the analyses, we employed the statistical software SPSS® version 17.0 (SPSS® Inc., Illinois, USA) and adopted a level of statistical significance below 5% ($p \leq 0.05$) in all comparisons.

Results

The group receiving non-surgical treatment included 72 patients: 23 (31.9%) underwent exclusive radiotherapy, 33 (45.8%) received chemotherapy concurrently with radiotherapy and 16 (22.9%) received induction chemotherapy followed by chemotherapy concurrently with radiotherapy; 8 patients (24.2%) in the concurrent treatment group and 7 (43.7%) patients who received induction did not complete all chemotherapy cycles. Of the 62 patients who received surgical treatment, 53 (85.48%) underwent total laryngectomy or pharyngolaryngectomy, whereas 9 (14.51%) underwent partial surgeries, and 38 (61.3%) were given adjuvant treatment (22 or 35.5% received exclusive radiotherapy and 16 or 25.8% received chemotherapy concurrent with radiotherapy). The mean follow-up time in the non-surgical group was 32 months versus 29 months in the surgical group.

The two groups were similar with regard to age and gender (p=0.430 and p=0.630, respectively). In the surgical group, there were 50 male patients (80.64%) with a mean age of 61.2±9.4 years, and 59 (81.94%) in the non-surgical group, with a mean age of 62.6±10.1 years. Considering all cases, the most common primary tumor site was the larynx (68.2%), with no significant difference between the two groups (p=0.682). Most patients had advanced tumors, with 28.3% T3 and 47.1% T4a. More advanced tumors were more frequent in the surgical group (p=0.004). Similarly, clinical stage IV was the most prevalent in both groups (52.9%), with the surgical group also being comprised of more patients in this stage (p=0.028).

About half (50.7%) of the patients did not have cervical metastasis at the beginning of treatment, 22.5% were classified as N2c and 13.4%, as N2b. The prevalence of cervical metastasis was not significantly different between the two groups (p=0.499). Alcoholism and smoking reached high rates, 88.8% and 60.5% respectively, with no difference between the groups (p=0.590 and p=0.560, respectively). The sampling and homogeneity data of the groups are detailed in Table 1.

Overall survival of all patients in the study was 79.2%, whereas disease-free survival was 70.6%, with four deaths in the surgical group versus eight in the non-surgical group (p=0.412). Recurrence or persistence of disease was found in 27 patients (20.1%): seven in the surgical group (25.9%, four regional recurrences, two isolated distant recurrences, and one recurrence classified as both); whereas 20 in the non-surgical group (74.1%; ten locoregional recurrences, six isolated distant recurrences, two local recurrences, and two concurrent recurrences – both locoregional and distant ones). In comparing disease-free survival between the two groups, we observed better results in patients who underwent laryngectomy, with 81.7% disease-free survival (seven events in 53 cases) as compared to 62.2% (20 events in 68 cases) in the non-surgical group (p=0.028).

In order to explore this difference, we went on to compare disease-free survival in the different clinical stages T and N between the two groups. Initially, we compared the two anatomical sites (larynx and hypopharynx), which might represent a selection bias in our study, but we found no significant differences in disease-free survival between the two groups (p=0.073). Thus, we kept the two sites grouped in the subsequent analyses.

TABLE 1 Demographic and clinical data of the sample and analysis of group homogeneity (n=134).

Variable	Total n (%)	Surgery n (%)	Non-surgery n (%)	p
Sex				0.630#
Male	109 (81.4)	50 (80.6)	59 (81.9)	
Female	25 (18.6)	12 (19.4)	13 (18.1)	
Age (years)*	61.4±9.8	61.2±9.4	62.6±10.1	0.430+
Comorbidities	63 (47.0)	28 (45.2)	35 (48.6)	0.690#
Primary site				0.682#
Larynx	90 (68.2)	42 (70.0)	48 (66.7)	
Hypopharynx	42 (31.8)	18 (30.0)	24 (33.3)	
Cancer staging (T)				0.004#
T1a	8 (5.9)	-	8 (11.1)	
T1b	10 (7.5)	3 (4.8)	7 (9.8)	
T2	15 (11.2)	6 (9.8)	9 (12.5)	
T3	38 (28.3)	14 (22.7)	24 (33.3)	
T4a	63 (47.1)	39 (62.9)	24 (33.3)	
Cancer staging (N)				0.499#
N0	68 (50.7)	29 (46.8)	39 (54.2)	
N1	13 (9.7)	7 (11.3)	6 (8.2)	
N2a	3 (2.2)	1 (1.6)	2 (2.8)	
N2b	18 (13.4)	6 (9.7)	12 (16.7)	
N2c	30 (22.5)	18 (29.0)	12 (16.7)	
N3	2 (1.5)	1 (1.6)	1 (1.4)	
Cancer staging				0.028#
I	18 (13.4)	3 (4.9)	15 (20.8)	
II	9 (6.7)	5 (8.0)	4 (5.6)	
III	26 (26.9)	15 (24.2)	21 (29.2)	
IV	71 (52.9)	39 (62.9)	32 (44.4)	
Smoking habit	119 (88.8)	54 (87.1)	65 (90.3)	0.560#
Alcohol abuse	81 (60.5)	39 (62.9)	42 (58.3)	0.590#

*Mean ± standard deviation; #Chi-square; +Student's t-test.

We then noted that, in stage III/IV patients, disease-free survival was higher in the surgical group (78.2%) compared to the radiochemotherapy group (55.5%, p=0.018). Patients were classified according to stage T: the oncological outcome in terms of the therapeutic modality used in patients with early-stage disease (T1 and T2) was not significantly different (surgical and non-surgical treatment, p=0.328). Yet, we found that, in advanced-stage patients (T3 and T4a), disease-free survival was higher in individuals who underwent surgical treatment (p=0.021). Additionally, when we compared survival between the two groups by dividing the patients according to neck staging, we found better oncological results for the surgical treatment in N0 or N1 neck cases (p=0.05). In N2 or N3 neck cases, in turn, there was no difference between the surgical and non-surgical groups (p=0.397). Comparative data for disease-free survival by different staging are shown in Table 2.

TABLE 2 Comparison of patients with squamous cell carcinoma of the larynx and hypopharynx who underwent surgical and non-surgical treatment for disease-free survival, stratified by site, staging, and T and N classification (separately and grouped).

Variables	Events/Total	Accumulated survival (%)	p (Log-rank)
Anatomical site			0.073
Larynx	12/82	77.8	
Hypopharynx	15/39	54.7	
Stage			0.398
Stage I/II			
Surgical group	0/6	100.0	
Non-surgical group	1/18	85.7	
Stage III/IV			0.018
Surgical group	7/47	78.2	
Non-surgical group	19/50	55.5	
T Classification			
T1/T2			0.328
Surgical group	0/7	100.0	
Non-surgical group	2/23	85.0	
T3/T4a			0.021
Surgical group	7/46	77.6	
Non-surgical group	18/45	54.2	
T4a alone			0.036
Surgical group	6/34	72.5	
Non-surgical group	11/23	44.2*	
N Classification			
N0 alone			0.043
Surgical group	0/25	100.0	
Non-surgical group	6/38	76.4	
N1 alone			0.029
Surgical group	0/4	100.0	
Non-surgical group	4/6	20.8**	
N0/N1			0.005
Surgical group	0/29	100.0	
Non-surgical group	10/44	68.7	
N2/N3			0.397
Surgical group	7/24	59.6	
Non-surgical group	10/24	51.2	

(*continues*)

TABLE 2 (Cont.) Comparison of patients with squamous cell carcinoma of the larynx and hypopharynx who underwent surgical and non-surgical treatment for disease-free survival, stratified by site, staging, and T and N classification (separately and grouped).

Variables	Events/Total	Accumulated survival (%)	p (Log-rank)
Grouped T and N classification			
T3 N0			0.266
Surgical group	0/6	100.0	
Non-surgical group	3/15	75.5	
T4a N0			0.036
Surgical group	0/13	100.0	
Non-surgical group	2/5	53.3	
T3/T4a N0			0.053
Surgical group	0/19	100.0	
Non-surgical group	5/20	70.0	
T3 N0/N1			0.142
Surgical group	0/8	100.0	
Non-surgical group	4/17	72.1	
T4a N0/N1			0.001
Surgical group	6/34	72.5	
Non-surgical group	5/8	29.2**	
T3/T4a N0/N1			0.004
Surgical group	0/23	100.0	
Non-surgical group	9/25	58.9	

*Median survival attained in 10 months.
**Median survival attained in 2 months.

When we compared the accumulated disease-free survival, grouping advanced T-stages and early N-stages (these patients had the best results with the surgical treatment; Table 2), we also observed better results in the surgical group, especially in the cases involving tumors T4a (T4aN0, p=0.036; and T4aN1, p=0.001).

In the multivariate analysis (Table 3), we found that the non-surgical treatment and the presence of N positive, especially N2/N3 nodal disease, were independent risk factors for disease progression (HR=3.76, p=0.017; and HR=11.82, p<0.0001, respectively). Furthermore, smoking was a factor associated with better progression, with lower persistence and disease recurrence rates (HR=0.08; p<0.0001). Once again, we found that the anatomic site, larynx or hypopharynx, was not an independent risk variable in this analysis (HR=1.31, p=0.589).

Discussion

Over the last few decades, there has been a shift in the treatment strategy for larynx cancer with advanced locoregional disease. There was an increase in the number of patients undergoing radiotherapy and chemotherapy and a decrease in the number of those treated with surgery.[5,10,11] According to the guidelines of the American Society of Clinical Oncology, disease management in association with larynx preservation was considered appropriate for most patients with T3 and T4 tumors without invasion into soft tissues through the cartilage.[12]

In our study, we found greater disease-free survival in patients with larynx or hypopharynx squamous cell carcinoma who were initially treated with surgery compared to those included in organ-preserving protocols. A multivariate analysis further corroborated this finding, in spite of the surgical group including patients with more advanced tumors. This clearly differs from some studies advocating conservative treatment in cases of advanced carcinomas of the larynx and hypopharynx.[2-6]

This difference was better characterized when contrasting the groups across the different T and N classifications. At this point, better oncological results were observed in patients with T3 and T4a tumors (mainly T4a) undergoing surgical treatment, which is partly in agreement with the literature.[10,13,14] Currently, what appears to be most generally accepted is that T4a tumors

TABLE 3 Multivariate analysis of risk related to disease relapse/persistence.

Variables	HR	95CI	p*
Age	0.99	0.95-1.03	0.599
Treatment modality			
Surgery	Reference		
Non-surgery	3.76	1.27-11.14	0.017
Anatomical site			
Larynx	Reference		
Hypopharynx	1.31	0.49-3.53	0.589
Presence of comorbidity			
No	Reference		
Yes	0.80	0.34-1.90	0.612
Cancer staging (T)			
T1/T2	Reference		
T3/T4a	2.89	0.62-13.61	0.178
Cancer staging (N)			
N0	Reference		
N+	6.49	2.17-19.37	0.001
N0/N11	Reference		
N2/N3	11.82	3.42-40.88	<0.0001
Cancer staging			
I/II	Reference		
III/IV	2.05	0.10-41.67	0.640
Smoking habit			
No	Reference		
Yes	0.07	0.02-0.22	<0.0001
Alcohol abuse			
No	Reference		
Yes	1.23	0.32-4.73	0.767

*Cox regression; NP = not performed due to the small number of cases.

(with coarse cartilage invasion and laryngeal extravasation) should be preferentially treated with surgery. Nevertheless, for T3 tumors, a course of conduct has not been well established yet.[15] Our study shows a trend towards better outcomes with surgery on intermediate tumors, which certainly encourages further studies.

As for lymph node status, when we stratified neck staging, we observed disease-free survival only in N0 and N1 cases, with better results in the surgical group as well. This was probably due to a worse prognosis inherent in the regional disease, diluting any benefit the surgical treatment could bring. In our study, the presence of cervical metastasis impacted on the decrease in disease-free survival also in the multivariate analysis.

Our main finding is the identification of laryngectomy as the best therapeutic modality for advanced tumors of the larynx and hypopharynx. In a first analysis, it may seem strange to group the two distinct sites in a joint analysis. Still, when dealing specifically with patients who have advanced disease with these topographies, it is not always possible to determine the epicenter of the tumor due to its bulky dimensions and/or involvement of multiple contiguous subsites. Furthermore, both the univariate and multivariate analyses showed, respectively, that the anatomical site (larynx or hypopharynx) was neither an associated nor a predictive variable with respect to the risk of recurrence or persistence of disease in these patients. Thus, we chose to keep the total sample in the subsequent analyses.

One of the limitations of the study is the relative heterogeneity of the groups because it is a historical cohort and not a randomized study. The surgical arm groups together patients who underwent total laryngectomy, pharyngolaryngectomy or partial laryngectomy, with or

without dissection, and with an adjuvant in some cases. The non-surgical arm, on the other hand, gathers exclusive radiotherapy, concurrent radiotherapy and chemotherapy, some cases of induction chemotherapy followed by radiotherapy and chemotherapy, and patients who did not complete the three cycles of concurrent chemotherapy. There are many different therapeutic modalities being compared, which makes it difficult to define the real benefit deriving from each one. Similarly, there is the inherent information bias of retrospective studies, which is in fact difficult to discriminate statistically. The selection of patients for each of the therapeutic arms may also be debated. However, it was mostly done at random as external referral of patients to our health care service. Still, the study has invaluably contributed to answering an essential question: is the current indiscriminate indication of organ-preserving protocols a sound practice in cases of moderately advanced larynx or hypopharynx tumor? The answer is no. It is essential that these patients be evaluated by a head and neck surgeon in an attempt to perform precise staging and provide an adequate definition of the treatment to be used multidisciplinarily. Certainly, further studies are required to define the exact cut-off point from which it is no longer possible or safe to attempt non-surgical treatment. Our study contributes to that end accordingly.

Based on three prospective studies that assessed 170 patients with advanced and resectable larynx or pharynx tumors, we evaluated the criteria for better indicating organ-preserving protocols. We created the acronym TALK (in Portuguese), according to which patients with advanced primary tumors (T4), low albumin level (< 4 g/dL), consumption of greater quantities of alcoholic beverages (> 6 doses/day – letter "L" for liquor) and lower Karnofsky indexes (< 80%) were the worst success results in preserving the larynx.[16]

In a multicenter retrospective study with 176 patients with larynx cancer, 65 were in clinical stage III, 51 underwent organ-preserving protocols, and 14 underwent laryngectomy. Of the 111 patients in clinical stage IV, 42 were given non-surgical treatment and 69 underwent total laryngectomy. Overall and disease-specific survivals at three years were 58% and 73%, respectively, for stage III and 42% and 53%, for stage IV. The choice of treatment did not appear to significantly influence survival for stages III (p=0.56) and IV (p=0.93). However, there was a trend towards better outcomes with surgical treatment, especially in patients with advanced disease.[17]

Interestingly, our results indicate that patients with a history of smoking had better oncological results in terms of disease-free survival compared to patients without such a history, irrespective of treatment modality. One hypothesis for this is patients that have larynx or hypopharynx cancer, even though they are non-smokers, must have other factors directly influencing their prognosis (immunosuppression, genetic predisposition and so on). Further in-depth studies are needed to evaluate this finding.

The impact of the treatment modality, particularly for advanced stage patients (stages III and IV), was studied by Bussu et al.[18] They retrospectively evaluated 166 patients with squamous cell carcinoma of the larynx treated with total laryngectomy, partial supracricoid laryngectomy or a combination of radiotherapy and chemotherapy. The organ preservation rate was 45% in the clinical group versus 76.7% for partial laryngectomy (p=0.0002). In T4a cases, they found improved survival in patients treated with total laryngectomy (78% vs. 68% for partial laryngectomy, and 54% for the combination of radiotherapy and chemotherapy at three years, p=0.031). These data corroborate the findings of our study for this group of patients.

No other non-surgical treatment has greater survival than the initial radical surgery.[1] Accordingly, our results are in agreement with what most studies in the literature show, which corroborates the thesis that the best treatment for moderately advanced and advanced tumors (T3 and T4a) is surgery followed or not by an adjuvant treatment for increased disease-free survival.

Conclusion

Patients non-surgically treated had poorer disease-free survival. Additionally, the presence of lymph node metastases was an independent risk factor for recurrence in both groups. Smoking was a factor associated with lower disease recurrence, irrespective of the treatment used.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Resultado oncológico de tratamento cirúrgico *versus* protocolo de preservação de órgão em câncer de laringe e hipofaringe

Introdução: A partir de estudos do início dos anos 1990, popularizou-se o tratamento não cirúrgico com radioquimioterapia, com a perspectiva de manutenção do resultado oncológico e preservação do órgão em pacientes com carcinoma espinocelular avançado de laringe e hi-

pofaringe. Entretanto, estudos posteriores demonstraram aumento da recorrência e da mortalidade com a difusão do tratamento não cirúrgico.

Objetivo: Comparar o resultado oncológico dos tratamentos cirúrgico e não cirúrgico de pacientes com câncer de laringe e hipofaringe e avaliar as variáveis associadas à recidiva de doença.

Método: Estudo de coorte retrospectiva de pacientes submetidos ao tratamento cirúrgico (laringectomia total ou parcial) e não cirúrgico (radioterapia isolada, radioterapia concomitante a quimioterapia ou quimioterapia de indução seguida de radioterapia e quimioterapia) de 134 pacientes, sendo 62 no grupo cirúrgico e 72 no não cirúrgico.

Resultados: As taxas de sobrevivência livre de doença foram maiores no grupo cirúrgico (81,7% *vs.* 62,2%; p=0,028), principalmente em estádios III/IV (p=0,018), tumores localmente avançados T3 e T4a (p=0,021) e casos N0/N1 (p=0,005). A presença de linfonodos cervicais, principalmente N2/N3, foi considerada fator de risco para recidiva de doença nos dois grupos (HR=11,82; IC95% 3,42-40,88; p<0,0001). Pacientes não submetidos ao tratamento cirúrgico apresentaram 3,8 vezes mais chance de desenvolvimento de recidiva (HR=3,76; IC95% 1,27-11,14; p=0,017).

Conclusão: Pacientes com câncer de laringe ou hipofaringe tratados de forma não cirúrgica tiveram menor sobrevivência livre de doença, especialmente nos tumores localmente avançados (T3 e T4a) e com pescoço pouco comprometido (N0/N1).

Palavras-chave: neoplasias laríngeas, neoplasias hipofaríngeas, carcinoma de células escamosas, laringectomia, radioterapia, tratamento farmacológico.

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The role of regulatory T cells, interleukin-10 and in vivo scintigraphy in autoimmune and idiopathic diseases – Therapeutic perspectives and prognosis

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Summary

Previous studies have demonstrated the expression of the CD25 marker on the surface of naturally occurring T cells (T_{regs}) of mice, which have a self-reactive cellular profile. Recently, expression of other markers that aid in the identification of these cells has been detected in lymphocyte subtypes of individuals suffering of autoimmune and idiopathic diseases, including: CD25, CTLA-4 (cytotoxic T-lymphocyte antigen 4), HLA-DR (human leukocyte antigen) and Interleukin 10 (IL-10), opening new perspectives for a better understanding of an association between such receptors present on the cell surface and the prognosis of autoimmune diseases. The role of these molecules has already been described in the literature for the modulation of the inflammatory response in infectious and parasitic diseases. Thus, the function, phenotype and frequency of expression of the α-chain receptor of IL-2 (CD25) and IL-10 in lymphocyte subtypes were investigated. Murine models have been used to demonstrate a possible correlation between the expression of the CD25 marker (on the surface of CD4 lymphocytes) and the control of self-tolerance mechanisms. These studies provided support for the presentation of a review of the role of cells expressing IL-2, IL-10, HLA-DR and CTLA-4 receptors in the monitoring of immunosuppression in diseases classified as autoimmune, providing perspectives for understanding peripheral regulation mechanisms and the pathophysiology of these diseases in humans. In addition, a therapeutic approach based on the manipulation of the phenotype of these cells and ways of scintigraphically monitoring the manifestations of these diseases by labeling their receptors is discussed as a perspective. In this paper, we have included the description of experiments in ex vivo regulation of IL-10 and synthesis of thio-sugars and poly-sugars to produce radiopharmaceuticals for monitoring inflammation. These experiments may yield benefits for the treatment and prognosis of autoimmune diseases.

Keywords: T_{reg} cells, IL-10, autoimmunity, idiopathies, scintigraphy.

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Introduction

The immune system is orchestrated by a complex network of components interconnected by cells with their various receptors, secreted mediators, expressed molecules, activated biochemical pathways and other components that, together and at different anatomical sites, enable the body to respond to different antigenic stimuli.[1,2] Generally, the responses are triggered by the interaction of exogenous antigens with the antigen-presenting cells, strategically addressed and responsible for antigen capture, transport and processing. Often, the defense strategy becomes permanent, conferring an immunological memory, capable of ensuring better response efficiency in later exposures.[1,2]

With the diversity of potential antigenic exposures, only a highly adaptive immune system is able to distinguish and respond to the various antigenic sequences found. The rationale for recognition of this system is the product of random recombination of gene segments that

generate lymphocytes with enormous receptor diversity. Such receptors characterize the cell phenotype of the lineage. The type and number of receptors are directly linked to the response to the different antigens (Ag).

The functions of the immune system include regulatory abilities, the mechanism responsible for ensuring that responses to antigens do not reach pathological and inhibitory levels, so that the immune system is not unduly activated against its own antigens, producing autoimmune disorders. Central tolerance is induced in the primary lymphoid organs, as a consequence of the recognition of autoantigens by immature T lymphocytes. To perform the proper protective function, multiple T-cell clones with wide antigen recognition diversity undergo a rigorous selection and thymic maturation process that occurs by recognizing their own peptides linked to major histocompatibility complex (MHC) molecules. The ability to distinguish between self and non-self antigens is defined as immunological tolerance and is critical to avoid intense self-recognition that can lead to pathological autoimmune responses. Therefore, autoreactive thymocytes that recognize autoantigens with high affinity are eliminated by clonal deletion in the thymus.[3,4] While this is an efficient mechanism, it is known that some autoreactive cells can dodge this barrier and leave the thymus, and can be activated in the periphery with potential to generate autoimmunity. The fact that autoreactive cells can be detected in the periphery clearly demonstrates that the thymic selection mechanism responsible for the elimination of autoreactive T cell clones is incomplete.[4,5] In this case, how can we ensure that such autoreactive cells will not be reactivated, promoting a break in tolerance and thus the emergence of autoimmune diseases? In other words, the immune system needs different, redundant features to ensure that potential autoimmune responses do not occur.

Peripheral tolerance mechanisms have been described in $CD4^+$ T cells and occur through anergy, clonal deletion and T cell suppression. Anergy may be induced during the Ag recognition process by T cells when: a) antigen presenting cells (APCs) do not express co-stimulatory molecules, thus rendering T cells incapable of responding to Ag; or b) when T cells express inhibitory receptors. In clonal deletion, there is repeated stimulation of T cells by antigens, resulting in cell death by apoptosis. The mechanism of suppression would be exerted by regulatory T cells (T_{regs}). T_{regs} represent a subpopulation of T lymphocytes characterized by the expression of $CD25^+$ molecules and the nuclear factor *Foxp3*. The *Foxp3* factor induces suppression of effector T cells, blocking the activation and function of these lymphocytes, thus being important in the control of the immune response to self and non-self antigens.[3] The activation regulation can best be understood from Figure 1.

It is possible that autoimmune disorders may be associated with failure to eliminate or inactivate high-affinity autoreactive cell clones during their ontogeny, and

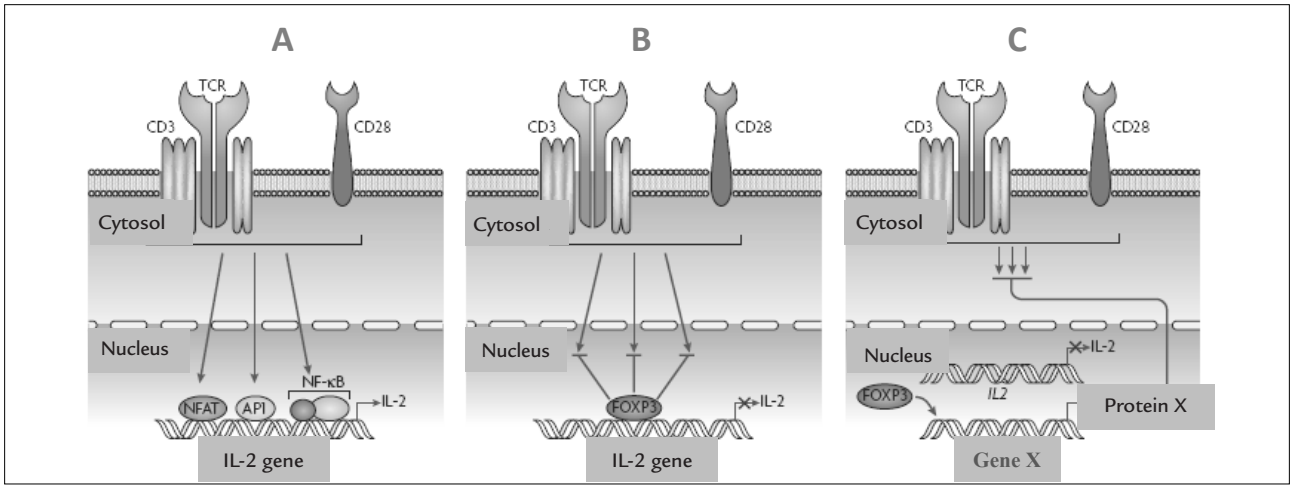

FIGURE 1 Regulation of T cell activation mediated by *Foxp3*. A. Signaling in effector CD4+ T cells. The binding of the T cell receptor (TCR) and the CD28 co-stimulatory molecule leads to the activation of the signaling pathways, resulting in the translocation of NFAT (nuclear factor of activated T cells) and AP1 (activator protein 1), with subsequent transcription of the IL-2 (interleukin 2) gene. B. Model of direct regulation of TCR mediated by *Foxp3* signaling. In this model, the *Foxp3* factor blocks TCR signaling through the inhibition of activation mediated by NFAT, NF[5]-κB and AP1. C. Indirect regulation model of TCR signaling: *Foxp3* factor modulates TCR signaling through the expression of a factor that can inhibit TCR-induced signals. (Adapted from Campbell and Ziegler.[6])

there may or may not be the failure of the immune system to control autoreactive intermediate affinity clones that have escaped to the periphery.[5,7] In this context, cells with a cellular response regulation function are fundamental and are also important in the modulation of the processes of eliminating pathogen and tumor antigen. These mechanisms occur with destruction of self tissues, exposure of autoantigens and production of pro-inflammatory cytokines, which, unless regulated, favor the induction and maintenance of autoimmune events. To exercise their function, the fundamental property of T_{regs} is the ability to: i) produce cytokines with the cellular response modulating function of TGF-β; and ii) induce cell-cell contact-mediated suppression. These soluble substances act in a complex network of regulatory mechanisms designed to ensure the modulation of immunological responses to the various antigens derived from infectious agents, tumors, autoantigens and allergens. Among T cells, several subpopulations exhibit regulation properties for exacerbated inflammatory response, such as IL-10-producing T_{regs}, which suppress some cytotoxic T cell responses in vivo,[5,7-10] including: CD8+CD28- T cells, $CD56^+$ T cells, γδ T cells[11-13] and $CD4^-CD8^-$ T cells.[13] In addition to T cells, there are other cell subtypes that have been described with such properties. IL-10-producing $CD1^+$ B cells are among them.[14]

Particularly among T_{regs}, there has been a strong emphasis on naturally-occurring T cells ($CD4^+CD25^+$ T cells), as described by Sakaguchi et al.,[15] which are potentially capable of suppressing activation, proliferation and/or effector function of $CD4^+$and $CD8^+$ T cells, and, possibly, NK cells, NK/T, B lymphocytes and dendritic cells.[15] T_{regs} are indispensable for the maintenance of tolerance mechanisms and knowledge of their functions is fundamental for understanding the pathophysiology of autoimmune diseases and to subsidize the strategies of interference in the mechanisms of recovery of tolerance in these pathologies. The need to review basic knowledge about $CD4^+CD25^+$ T cells, their role in different rheumatic diseases, and the prospects of advancement in the treatment of autoimmune diseases through the manipulation of these cells is therefore justified.

Naturally occurring T cells are related to the maintenance of self-tolerance and are very important for the maintenance of homeostasis of the immune system.[16] T_{regs} are involved in the inhibition of the activation and expansion of autoreactive lymphocytes in the peripheral tissues and present an inhibitory capacity with a proven role in the negative regulation of the immune response also against exogenous antigens and autoantigens.[17-19] Currently, T_{regs} have been investigated for their role in the immunomodulation of responses in inflammatory, neoplastic, autoimmune syndromes and also in transplant rejection, in the hope of opening other therapeutic perspectives to control exacerbated immune responses without the induction of anergy or nonresponsiveness, but by activating cellular function.[20-23] Early reports on cell subtypes specialized in regulating the immune response occurred in the 1970s, when it was shown that some subtypes of T lymphocytes were able to suppress the development of autoimmune diseases.[24] Later, other authors[1,15] demonstrated the constitutive labeling of the α-chain receptor of IL-2 (CD25) by $CD4^+$ T lymphocytes and attributed to it a role in the suppression of autoimmune diseases in mice. Proof of this role was possible by the removal of $CD25^+$ splenocytes in healthy rodents, triggering autoimmune disorders such as thyroiditis, insulinitis, polyarthritis, glomerulonephritis, and graft versus host disease. It was also demonstrated that adoptive transfer of this population inhibited autoimmunity in experimental models.[15,25]

Interest in the study of T_{regs} is due to the key function of this cellular population in the maintenance of the mechanisms of self-tolerance and in the regulation of the immune response.[20] $CD4^+CD25^+$ T lymphocytes represent 5 to 10% of total $CD4^+$ cells in peripheral blood.[25-27] Evidence obtained in later studies shows that $CD4^+CD25^+$ thymocytes are selected in the thymus from interactions with proper peptides presented by MHC-II molecules.[27] Positive selection of these cells depends on high-affinity interactions with autoantigens expressed on MHC molecules.[28] The mechanism by which $CD4^+CD25^+$ T cells escape negative selection is still controversial, but it is believed that these, once positively selected through high affinity recognition of their own peptides, produce anti-apoptotic molecules that protect them from negative selection.[29] T_{reg} cells, besides the thymic generation, can be induced in the periphery by the action of specific soluble factors on naïve cells that have just left the thymus.

Annunziato et al.[30] evaluated phenotypic and functional characteristics of human thymus cells and have demonstrated that these cells respond to chemotactic signals from macrophages and epithelial cells constitutive of the thymus itself, and that are capable of expressing $CD4^+$, $CD25^+$ and mTGF-β1, as well as molecules directly with immunosuppressive function, such as CTLA-4. These cells had low production of IL-10 and none of IL-2, IL-4, IL-5, IL-13 and IFN-U.[30,31] In addition to thymus, human T_{reg} cells were isolated in other microenvironments, such as secondary lymphoid organs, e.g., tonsils and spleen, as well as umbilical cord blood.[1] Also, $CD4^+CD25^+$ T cells

present in the thymus have been reported as naïve cells that become activated and express a memory phenotype when they exit toward the periphery.[32]

Studies by Sakaguchi et al.[15,16] had already characterized the T-cell phenotype based only on the constitutive expression of the CD4 and CD25 markers, although it is known that any other $CD4^+CD25^-$ cell may, after being activated, begin to transiently express the CD25 molecule. In humans, $CD4^+$ T cells have differentiated profiles of CD25 receptor expression, with differentiated intensities detected in the medium channel of fluorescence, so that it is possible to identify, in the "gate" in $CD4^+CD25^+$ cells, a more abundant population, expressing low levels of CD25, and a lower percentage of CD4, with high intensity of expression of this receptor.[27,33] This last population, with intense expression of CD25, corresponds to the pool of this subpopulation. However, there is limitation of the CD25 receptor as a T_{reg}[20] phenotypic marker. The current strategy for isolation and characterization of T_{regs} is based on the recognition of this marker. CD25 also represents, in the physiology of this cell, an indispensable component for its generation and maintenance in the organism.

As previously mentioned, regarding the biomolecular approach, researchers[6,15] have demonstrated that transcription factor *Foxp3* is predominantly expressed by thymic and peripheral T_{regs}.[6,15] Naïve T cells transfected with *Foxp3* mRNA acquire characteristic of regulatory cells becoming anergic and suppressive in vitro. It was further observed that the transfected cells acquired T_{reg}-like phenotype in relation to phenotypic expression and the production of cytokines and other T-related molecules such as CD25, CTLA-4, CD103 and GITR. Transfected cells also have the ability to suppress the proliferation of other T cells and to inhibit the development of autoimmune disease and inflammatory vessel disease in vivo.[6,34] It has also been shown that the number of T_{regs} is increased in mice transgenic to *Foxp3* and that mice KO for this gene show hyperactivation of T cells. According to previous reports,[6,15] *Foxp3* appears to be a very important gene in the development and function of $CD4^+CD25^+$ T cells, both in mice and humans.

Patients with *Foxp3* mutation have been shown to develop IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). This condition consists of an autoimmune disorder that affects multiple organs with development of allergy and inflammatory vessel disease. These patients appear to be impaired in terms of the development of T_{regs}, thus presenting defective suppression function, which induces a state of hyperactivation of T cells that become reactive against autoantigens, commensal bacteria of the intestine or innocuous environmental antigens.[20] Most T cells expressing *Foxp3* are $CD4^+$ $CD25^{+high}$ and $CD4^+CD25^{+low}$, that is, cells with high expression of CD25 and cells with low expression of CD25 that are capable of suppressing T cell proliferation with the same intensity.

Later, Seddiki et al.[35] demonstrated that anti-CD127 monoclonal antigen is able to clearly mark the population of T_{regs}, with suppressive activity. Previous studies have reported that *Foxp3* expression did not always correlate with the expression of the CD25 molecule.[36] Liu et al.[37] found that most $CD4^+Foxp3^+$ cells were $CD25^{high}CD127^{low}$. This study demonstrated that CD25CD127 labeling was able to accurately indicate a population of suppressor T cells with a higher degree of purity, leading to the assumption that $CD4^+$ T cells could actually be significantly higher than previously thought. Thus, it is possible to distinguish clearly from a population of T cells the newly activated effector cells and memory cells, since only newly activated T cells have constitutively low expression of CD127, whereas memory cells have high expression of this marker and the traditional effector cells rapidly re-express this marker upon activation.[35-37] In addition, the use of other markers such as CTLA-4 and CD122, although also expressed under activation conditions, may aid in their characterization. In autoimmune disorders such as rheumatoid arthritis (RA), there is a lot of evidence that the breakdown of immune tolerance mechanisms begins in the thymus with the escape of clones with self-reactive potential. In our preliminary studies, we demonstrated that the majority of the peripheral blood samples from RA patients who were evaluated had the HLA-DR marker. It is known that certain HLA-DR alleles determine both the susceptibility to the disease and its severity. These determinants, and perhaps others of a genetic nature, may be susceptible to an unidentified environmental factor. Nevertheless, progression or not to autoimmunity appears to be critically and relevantly determined in the periphery. Most often, tolerance mechanisms can control the peripheral activation of autoreactive clones that are eliminated or anergized. When this control is insufficient, autoimmune disease manifests itself.

T_{reg} cells are responsible for the maintenance of "active" mechanisms of suppression and immunoregulation that work together with the other mechanisms of peripheral tolerance. Several studies have been carried out, evaluating the role of T cells in the maintenance of peripheral tolerance and the pathophysiology of autoimmune diseases. Its relevance in this process has been clearly demonstrated in murine models in which the absence or depletion

of T cells triggers systemic autoimmune diseases with high titers of antinuclear antibodies as well as autologous organ-specific antibodies.[15] Important findings, such as defects in function, phenotype and frequency of immunoregulatory cells, have been reported in several human autoimmune rheumatic diseases, thus evidencing their important role in maintaining immunological tolerance and in the pathophysiological mechanisms of these diseases. The proportion of T_{reg} cells in peripheral blood was related to the observation of increased levels in peripheral blood and synovial fluid, in addition to the demonstration of suppressive activity more powerful than that observed in the peripheral ones.[38-40] In contrast, normal levels of T_{reg} in peripheral blood were also detected in some studies. This variability probably stems from differences in disease stage, therapy and certainly variations in the strategies for characterization of RAs.

In more recent research, Cao et al.[41,42] found that in approximately 95% of patients with rheumatic diseases that progress with arthritis, such as: RA, juvenile rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus (SLE), Behcet's disease, rheumatic polymyalgia and mixed connective tissue disease, all presenting high levels of T_{regs} in the inflamed joint, despite the clinical condition and disease time.[41,42] These authors suggest that T_{regs}, even if numerically enhanced in inflamed synovium and with normal suppressor function, are unable to suppress secretion of proinflammatory cytokines by activated T cells or monocytes. This may occur because the suppressive action of T lymphocytes would be overcome by other lymphocytes with strong activation signals present at these sites, including Th1 and Th17 cells.[43] This fact is in accordance with the data presented by Nistala et al.,[44] who demonstrated that the balance between the populations of T_{regs} and Th17 is inversely correlated and very important in the progression of the disease.[44] Failure of the T_{regs} in RA was also suggested by Van Amelsfort et al.[45] who reported levels and suppressive activity of this increased cellular subtype in synovium of RA patients compared to the same peripheral blood population. However, inflammation persisted. Monocyte-derived cytokines, such as TNF and IL-7, as well as co-stimulatory molecules such as CD28, are possibly counteracting factors in the suppression of T_{regs} in these patients, both in synovial fluid and in peripheral blood, preventing suppression.[45]

Interleukin 10 (IL-10) in autoimmune and idiopathic diseases

In earlier preliminary studies, the authors observed that in myasthenia gravis (MG), an autoimmune disorder, there is an increase in T_{reg} cells in the blood of individuals not treated with corticosteroids, and a decrease in $CD8^+$ T cells. The population of $CD4^+IL\text{-}10^+$ T cells obtained by Ficoll gradient separation and fluorescently labeled with monoclonal antibodies was significantly increased, with a significant reduction of clinical symptoms. In this case, IL-10 in association with CD25 appears to exert peripheral tolerance control. Studies by Falcão et al.[46] had already demonstrated a role of this cytokine in controlling exacerbated responses in infectious-parasitic diseases. In schistosomiasis mansoni, asymptomatic patients have a high IL-10 profile, together with other molecules such as HLA-DR and other co-stimulatory molecules. However, in another study, the authors reported that T_{regs} did not significantly express INF-γ and inhibition of T cell proliferation was not achieved. Studies have shown that IL-10 inhibits APC activation and is related to inflammatory control reactions in target tissues.[47]

In MG, a disease that attacks the postsynaptic portion of the neuromuscular junction and is characterized by fluctuating muscle weakness, the biological heterogeneity investigated for the first time when rabbits with acetylcholine receptors were purified to obtain antibodies against that receptor has been demonstrated. Immunized rabbits had fallen ears and palpebral ptosis (drooping eyelid) with improvement at rest and worsening with exercise, infections and emotional stress.[48] The role of these antibodies in the etiology of MG was clearly established in the 1970s, when plasmapheresis proved to be effective in the removal of antibodies and consequent functional improvement for more than 2 months.[49,50] Well-established anatomical changes were also observed, including increased neuromuscular junction size and decreased post-synaptic membrane length. Other important observations have been reported on the role of autologous antibodies in MG, since approximately 50% of patients with the disease without *Ach* anti-receptor antibodies have antibodies against a muscle membrane enzyme called muscle-specific tyrosine kinase (anti-Musk). Lavrnic et al.[48] analyzed 17 patients with this condition, observing a higher prevalence of women, predominant facial and bulbar involvement and refractoriness to anticholinesterase compounds. Because it is an autoimmune disease, other conditions of the same nature may coexist in a patient with a diagnosis of MG, and should be screened rationally, especially hypothyroidism, hyperthyroidism and thymus disease.[50] Seventy percent (70%) of patients have thymic hyperplasia and approximately 10% have thymoma – with potential for malignant behavior, which is more common in patients aged 50-70 years.

As previously mentioned, patients with clinically controlled RA had an increased T_{reg} profile; however, the authors found a differential expression of receptors on the surface of peripheral blood lymphocytes from individuals with a diagnosis of MG, with a symptom of muscle weakness, and who were treated with prednisone and azathioprine, which are immunosuppressive agents. They showed a decreased profile of T_{regs} and $CD4^{+}$IL-10, as well as increased $CD8^{+}$CTLA-4^{+} T cells.[51]

As a consequence of previous studies,[52] the authors also reported the role of these two receptors in idiopathic diseases, such as Bell's palsy. Patients with autologous induction of IL-10, obtained through receptor purification, have been shown to have clinical improvement as well as increased T_{reg} expression. $CD4^{+}$ T lymphocytes were found to present increased expression after one week of induction. Paralysis affects the facial nerve (cranial nerve VII), which results in inability to control the facial muscles on the affected side. Several other conditions can also cause facial paralysis, for example, brain tumor, stroke and Lyme disease. A person may experience pain behind the ear a few hours before muscle weakness occurs. Clinical treatment includes prescribing anti-inflammatory drugs such as prednisone. Also, as in MG, immunosuppressive treatment has been used and is effective in controlling symptoms and reducing exacerbations. Pyridostigmine is reserved for refractory cases. The different dosages of glucocorticoid (daily use, alternating use or pulse therapy) do not seem to yield different efficacies.[53-55] The receptor-glucocorticoid complex enters the cell nucleus and causes some changes in the DNA that stimulate or repress the synthesis of certain tissue proteins. Prednisone is particularly effective as an immunosuppressant and alters the performance of the immune system, with a decrease in mediators for inflammation. This decrease, in certain cases, prevents the communication with other cells of the immune system that should be recruited in order to modulate the inflammatory process through the production of physiological proteins, such as interleukin 10 (IL-10). Prednisone is used in autoimmune and inflammatory diseases and, at a given moment, induces immunological immunosuppression states, precisely because it prevents the receptors fixed on the surface of the defense cells and soluble cofactors from playing their roles in cellular activation, considering that the analogous receptors of the drug can share the same ligands of IL-10, preventing its action. Prednisone is biotransformed in the liver into prednisolone by the action of the enzyme dehydrogenase 11-beta-hydroxysteroid type 1. From 1 to 3 hours after administration, the drug reaches plasma peaks. Its plasma half-life is approximately 3 hours, its biological half-life thus being 12 to 36 hours in this case.[56]

Receptors are surface proteins that bind to external signaling molecules of high affinity cells and convert this extracellular event into one or more intracellular signals that alter the behavior of the target cell. Note that the receptors for IL-10 are arranged as two-chain α-tetramers (IL-10 receptor α-chain) and two β-chains (IL-10 receptor β-chain) (Figure 2). Signaling occurs through interaction with Janus kinases. IL-10 belongs to these two receptor chains, which associate the *Jak-1* and *Tyk-2* kinases of the Janus family. *STAT-3* is the main "downstream" signaling molecule induced by IL-10, which is produced mainly by regulatory T cells but also by macrophages and keratinocytes present in epithelial tissue.[1] *STAT-3* is expected to act to inhibit gene transcription of inflammatory and/or autoreactive receptors, forming the *STAT* complex in association with *Jak-1* and *Tyk-2*, with consequent nuclear translocation and gene activation, since mRNA of these enzymes were detected by RT-PCR, with bands of around 120KDa, with anti-janus-kinase1. The high expression of $CD4^{+}CD25^{+low}$ and $CD4^{+}IL\text{-}10^{+\ high}$ T cells found after induction of autologous IL-10 can be explained by occupying both R1 and R2 IL-10 receptors. Also, in many cases, the generation of autoreactive antibodies or T cells can also be attributed to the role played by infectious agents present in the body of the individual, such as bacteria, which lead to the generation of antibodies and T cells which, in turn, react with many different epitopes of the infectious organism. If one of these antigens is similar to an autoantigen it may result in an autoimmune response.[52]

Ex vivo monitoring of IL-10 can be obtained by analysis of human peripheral blood mononuclear cells, grown in vitro and induced by blastogenesis for the production of proteins (interleukins), through mitogenic stimulation with PHA (phytohemagglutinin). Protocols developed for in vitro and in vivo experimental phase were filed with patent application PI0206722-6, supported by experiments with animal models highly homologous to the human genome.[52] The protein fraction of IL-10 obtained by PCR and purified, free of contaminants can be analyzed by electrophoresis and quantified by UV-visible spectrometry. The procedure may become a routine.

Falcão et al.[52] demonstrated that IL-10 suspensions, ex vivo, can be applied at the inflammatory site, connective tissue and muscle. In cases of syndromes that render the synapses between first-order neurons in the periphery unfeasible, the application was close to the areas of muscle, subcutaneous or intradermal flaccidity.[52] Assuming a regular interval of 10 days between applications, the monitoring

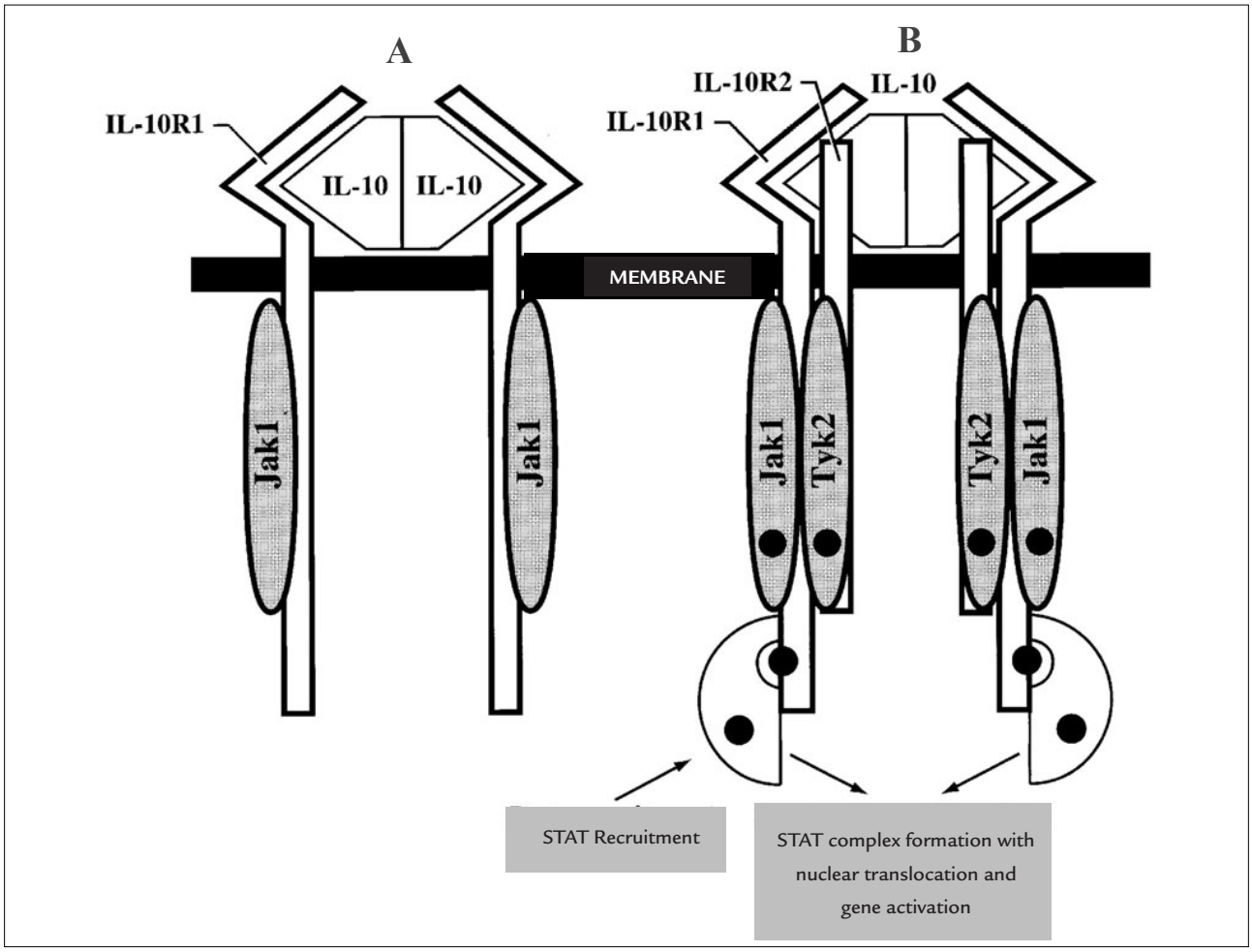

FIGURE 2 IL-10 receptor binding via *STAT-3*. A. Binding of IL-10 to IL-10R1 receptor via receptor-anchored *Jak-1* kinase. B. Binding of IL-10 to IL-10R2 receptor, recruitment of *STAT-3* and *STAT* complex formation and gene activation by *Jak-1* and *Tyk-2* kinases. Both "outside-in" and "inside-out" signaling are associated with distinct conformational changes in the extracellular segment. These changes vary with the type and nature of the ligand and are modulated by divalent cations. (Adapted from Abbas and Lichtman,[1] 2005.)

of the modulation of the inflammatory profile was performed according to Falcão et al.[52] The receptors were identified by flow cytometry, and their fluorescent histograms were prepared so that the absolute number of cell surface receptors labeled with the anti-receptor fluorescent monoclonal antibody of interest was generated, or the mRNAs for genes of the receptors were detected. As a result, they found an increase in the production of $T_{regs,}$ $CD4^{+}HLA\text{-}DR^{+}$, a decrease in $CD8^{+}CTLA\text{-}4^{+}$ and an increase in the expression of IL-10 by T_{regs} up to the fourth week, with a mean of the absolute number of receptors maintained after the sixth week. The expression of $CD8^{+}INF\text{-}\gamma^{+}$ and $CD14^{+}INF\text{-}\gamma^{+}$ was markedly decreased in the samples evaluated. [52]

Thus, it is conclusive that the monitoring and manipulation of proinflammatory interleukins has the potential to assist in the prognosis of anti and pro-inflammatory and degenerative changes in situ, monitoring the course of the disease.

Synthesis of tracers for in vivo monitoring

Image monitoring of symptoms of autoimmune diseases, such as RA, is preferable considering that such a technique will directly contribute to the accuracy of the diagnosis and consequently the establishment of the therapeutic mode and its intensity.[57] The accurate definition of the site with a design of the inflammatory focus is relevant in the choice of therapeutic management in RA.[58] Radiological imaging, radiography, computed tomography, nuclear magnetic resonance or ultrasound may favor an analysis of the deleterious effects on the anatomical structures in the peripheral joints.[59] However, such images do not aid in the early analysis of RA. Scintigraphy, on the

other hand, may promote an early diagnosis of inflammatory processes by monitoring the early stages of inflammation. Thus, radioisotope scintigraphy is expected to contribute to the diagnosis of RA by monitoring functional and physiological changes at the inflamed site before anatomical structural changes consequent to RA can become apparent.[60]

Positron-emitting fluoride-18-labeled deoxy-glucose (FDG) is a radiopharmaceutical used in positron emission (PET) scintigraphy. The compound accumulates in the inflammatory site, given the high local metabolism. The high supply of leukocytes in the inflamed site leads to increased glucose consumption.[61] However, due to the high cost of production of this 110-minute half-life radiopharmaceutical, together with the cost of PET imaging, it is currently impracticable to perform systematic clinical studies of RA using this technique. Cost reduction or new methods and radiopharmaceuticals should be produced to enable scintigraphy of RA.[61] The use of radiolabeled ex-vivo leukocytes is attractive; however, they involve difficult management with high control of sterility and apyrogenicity.[62] Although leukocyte scintigraphy radiolabeled with ^{111}In and ^{99m}Tc is a gold standard for the diagnosis of inflammation, the process of marking autologous leukocytes with ^{99m}Tc-HMPAO demands manipulation of blood samples in aseptic facilities with the reintroduction of these samples into the patient.[63] Obviously, there is the inherent risk of contamination, during manipulation of PBMC cells and isolation and labeling of leukocytes.[63]

A recent patent PI0904754-9, developed by the research group coordinated by the author, has shown that Tc-99m-labeled thio-sugar analogues of glucose are efficient in detecting inflammations.[64] Previous synthesis studies had been successfully performed using 5-thio-D-glucose; however, due to cost issues, there was a need to replace the thio-sugar molecule.[65] The importance of thio-sugars in inflammations was demonstrated in the temporomandibular joint (TMJ) of rats.[63] The patent involves 5-thio-glucose and 1-beta-thio-D-glucose labeled with Tc-99m.[64] The results show significant differences in the uptake of ^{99m}Tc-1-TG in the inflamed TM joint compared to the control, with high renal excretion. Tc-99m-labeled glucose analogs may become radiopharmaceuticals important for detection in the monitoring of inflammations such as AR due to the low cost and high technological feasibility. However, despite the murine investigations, there is still a need for clinical investigations demonstrating its efficiency in the early detection of RA and the degree of disease involvement in humans before and after immunological treatment.

Research on the synthesis and characterization of sugars with heavy metals has advanced. Recently, Dalmazio and Campos[66] demonstrated by mass spectrometry the viability of direct labeling of sugar polymolecules with Sm, Gd, B, Li, Tc, Sm, Ho, Eu, and other elements. These metal-sugar complexes make it possible to define several tracers for different modalities of medical imaging tests. These studies lack in vivo experimentation, but already offer a promising perspective in the monitoring of autoimmune diseases.

IL-1 and IL-6 interleukins play a crucial role in RA and osteoarthritis in the early processes of cartilage breakdown and destruction.[65] A significant increase of IL-6 in patients with osteoarthritis was identified by Kaneyama et al.[67] In 2014, in turn, Sukedai et al.[68] report the relation between TNF-α and cartilage degeneration. These authors show that IL-8 is closely involved with the acute phase of the inflammatory process. Thus, interleukins, such as IL-1, IL-6, IL-8, are proteins with which in vivo monitoring may lead to differential diagnosis of RAs.

Radiolabeled sugars serve the monitoring of inflammation induced by autoimmune diseases; however, they are not specific. It is worth saying that the interleukins themselves have high potential for radiolabeling. Rennen et al.[61,69] performed the labeling of IL-8 with Tc-99m making it possible to diagnose inflammation through radiolabeled interleukins.[69] Thus, we conclude that inflammatory cytokines are potential markers to aid in the diagnosis and prognosis of anti- and pro-inflammatory and degenerative changes in situ, monitoring the course of the disease. Radiolabeled cytokines, together with high metabolism labeling radiopharmaceuticals, represent a promising class of compounds for the evaluation of autoimmune diseases, since these proteins play an important role in inducing and maintaining the disease process.

Conclusion

The present review addressed cellular markers whose analysis and modulation may be useful in the treatment of autoimmune and idiopathic diseases, as well as in the prognostic monitoring of diseases. It has been noted that the ex-vivo monitoring and manipulation of interleukin IL-10 is relevant for treatment, and that thio-sugars, monossaccharides, polysaccharides and radiolabeled interleukins are tools for in vivo monitoring of autoimmune and idiopathic diseases. Future consolidation of scintigraphic methods can help monitor the progression of such diseases. Advances in research on modulation and generation of radioactive drugs involving cell markers for diagnosis and therapy may bring benefits to patients with autoimmune diseases.

Resumo

O papel de células T regulatórias, da interleucina 10 e da cintilografica *in vivo* em doenças autoimunes e idiopáticas – Perspectivas terapêuticas e prognóstico

Estudos anteriores já haviam demonstrado a expressão do marcador CD25 na superfície de células T de ocorrência natural (T_{regs}) de camundongos, que apresentam perfil celular autorreativo. Recentemente, foi detectada, em subtipos de linfócitos de indivíduos acometidos por doenças autoimunes e de causa idiopática, a expressão de outros marcadores, que auxiliam na identificação dessas células, entre os quais: CD25, CTLA-4 (*cytotoxic T-lymphocyte antigen 4*), HLA-DR (*human leucocyte antigen*) e Interleucina 10 (IL-10), abrindo novas perspectivas para a melhor compreensão de uma associação entre esses receptores presentes na superfície celular e o prognóstico de doenças autoimunes. O papel dessas moléculas já havia sido descrito na literatura na modulação da resposta inflamatória em doenças infectoparasitárias. Dessa forma, foram investigados a função, o fenótipo e a frequência de expressão, do receptor de cadeia α da IL-2 (CD25) e de IL-10 em subtipos de linfócitos. O modelo murino tem sido utilizado para demonstrar uma possível correlação entre a expressão do marcador CD25 (na superfície de linfócitos CD4) e o controle dos mecanismos de autotolerância. Essas pesquisas forneceram suporte para apresentação de uma revisão sobre o papel das células que expressam os receptores de IL-2, IL-10, HLA-DR e CTLA-4 no monitoramento da imunossupressão, em doenças de classificação autoimune, abrindo perspectivas para o entendimento dos mecanismos de regulação periférica e sobre a fisiopatologia dessas doenças no ser humano. Além disso, é discutida como perspectiva uma abordagem terapêutica fundamentada na manipulação do fenótipo dessas células, bem como de modos de monitoramento cintilográfico das manifestações dessas doenças, por meio da marcação de seus receptores. Nestes, foram incluídas descrições das experiências em regulação *ex-vivo* de IL-10; de síntese de tioaçúcares e de poliaçúcares para produção de radiofármacos para monitoramento de inflamações. Essas experiências podem trazer benefícios na terapia e no prognóstico de doenças autoimunes.

Palavras-chave: células T_{regs}, IL-10, autoimunidade, idiopatias, cintilografia.

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Sickle cell retinopathy: A literature review

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Summary

Hemoglobinopathies are a group of hereditary diseases that cause quantitative or qualitative changes in the shape, function or synthesis of hemoglobin. One of the most common is sickle cell anemia, which, due to sickling of erythrocytes, causes vaso-occlusive phenomena. Among the possible ocular manifestations, the most representative is retinopathy, which can lead to blindness if left untreated. Therefore, periodic ophthalmologic monitoring of these patients is important for early diagnosis and adequate therapeutic management, which can be done localy by treating the lesions in the eyes, or systemically.

Keywords: retinal diseases, anemia, sickle cell, review, hemoglobinopathies.

Introduction

Sickle cell disease is the most frequent and disabling chronic hemolytic anemia in our country. Sickle cell anemia is characterized by the production of abnormal hemoglobins that deform and stiffen red blood cells, causing increased blood viscosity and microcirculation occlusion to varying degrees.[1] These hemoglobins are insoluble at low oxygen concentrations, and tend to crystallize.[2] Sickle hemoglobin (HbS) is characterized by a mutation in the β-globin gene involving a single nucleotide (GAG → GTG) which replaces glutamine with valine in sixth amino acid position.[3,4] The most common genotypes are homozygous (SS), heterozygous or sickle cell trait (SA), hemoglobin C trait (SC), hemoglobin D trait (SD), and thalassemia genotype (S-Thal).[1,5-8]

The World Health Organization (WHO) estimates that more than 5% of the world population has some type of hemoglobinopathy. The estimate of new cases in Brazil is 700 to 1,000 per year, with a prevalence of over 2 million carriers of the HbS gene.[9] Sickle cell retinopathy, the subject of this study, develops in up to 42% of sickle cell individuals in the second decade of life.[10]

The systemic manifestations of sickle cell disease may be neurological, ophthalmologic, cardiac, pulmonary, gastrointestinal/hepatobiliary, renal/genitourinary, splenic, muscular/skeletal, and growth and developmental disorders.[11] They are more severe in homozygotes for cell disease (SS) than in heterozygotes with sickle hemoglobin C (SC), and yet visual loss due to proliferative retinopathy is more common in the latter.[6]

Sickle cell retinopathy is not frequently reported in the literature, and studies in this regard are very old. This was one of the motivations for our study, which seeks to gather the information known and thereby clarify the progression of the disease, its diagnosis and treatment.

Method

We searched the PubMed (US National Library of Medicine – National Institutes of Health) database using the following keywords: "retinopathy," "sickle cell," "sickle cell disease" and "sickle cell anaemia."

Ocular manifestations

Ocular manifestations of sickle cell anemia include orbital, conjunctival, uveal, papillary, and especially retinal changes.[5,7,12] Retinal changes characterize sickle cell retinopathy, which may be non-proliferative or proliferative and is divided into five stages.[1,10,12,13]

Among the forms of sickle cell anemia, SS patients present a more severe systemic clinical picture than those with type SC. On the other hand, occlusive ocular effects are more predominant in SC patients, who present only moderate anemia and higher blood viscosity.[5] These vaso-occlusions occur primarily in younger people, and are first

observed at the periphery of the retina, resulting in unperfused and presumably ischemic areas. Retinal neovessels, in turn, tend to develop in these areas, but not necessarily.[14] Individuals with SCD as well as sickle cell trait (AS) are at increased risk of developing increased intraocular pressure when they present with hyphema (bleeding in the anterior chamber of the eye).[12] Low fetal hemoglobin (HbF) is generally associated with increased intravascular sickling and is responsible for several vaso-occlusive complications in homozygous SS.[15] In addition, Roy et al. associated proliferative retinopathy with low levels of HbF.[16]

Another possible change cited by Ballas et al.[11] is glaucoma, due to high ocular pressure caused by clogging of the trabecular meshwork and the inflow of aqueous humor. Vaso-occlusion leads to optic nerve damage, causing visual impairment even before the occurrence of retinal changes.[17]

Non-proliferative retinopathy can occur with small intra-retinal hemorrhages, possibly due to ischemic vessel wall necrosis, called salmon patches; the bleeding then becomes yellow and then white, disappearing without a trace. There may also be hyperpigmented lesions in deeper or sub-retinal hemorrhages, called black sunbursts (Figure 2).[12] Maculopathy occurs as a result of chronic changes in the perifoveal capillary network.[8]

According to David et al.,[1] there was macular change in 14.4% of the patients. However, Clarkson found 4.6% of this same type of lesion. Both found higher prevalence in the SS type.

Although proliferative retinopathy has the same genesis as nonproliferative retinopathy, their progression differs. It was divided by Goldberg in five stages,[12,18] correlating them with their order of appearance: Stage I is characterized by definitive arteriolar occlusion, with consequent retinal hypoxia and rearrangement of adjacent capillaries. In the next stage (stage II), the budding of new vessels begins, with possible dilatation, aiming to join the vascular and avascular retina. In stage III, under the action of angiogenic events, pre-retinal neovascularization occurs, forming the so-called retinal sea fans.[12] These new vessels develop from arteriovenous loops or crossings, and often undergo self-infarctions probably caused by the unusual characteristics of the flow. The new vessels are fragile, immature and adherent to the vitreous. This facilitates the occurrence of vitreous hemorrhage and characterizes stage IV of proliferative retinopathy in sickle cell disease. When bleeding reaches the visual axis, it causes scotomas and amaurosis. The repetition of these hemorrhagic phenomena leads to rupture, retinal detachment and vision loss (stage V), the final stage of sickle cell proliferative retinopathy.[12]

Sickle cell retinopathy develops in up to 42% of sickle cell individuals in the second decade of life.[10] Vascular tortuosity is the most common finding (Figure 1), reported by the authors in about 30-50% of cases.[5,19,20] Cury et al.[3] found a prevalence of 19.6%, a result that may be justified by the fact that the study was conducted in children only. In addition, about 10-20% of patients will develop proliferative retinopathy,[8] mainly in the fourth and fifth decades of life.[21]

Diagnosis

In the early stages, the disease is asymptomatic, and meticulous ophthalmologic monitoring should be performed.[8] Diagnosis is made by retinography and fluorescein angiography in cases with fundoscopic alterations, as well as measurement of visual acuity and intraocular pressure.[5,7,8,13]

Treatment

Treatment is performed in different ways, including diathermy, cryotherapy and argon or xenon photocoagulation.

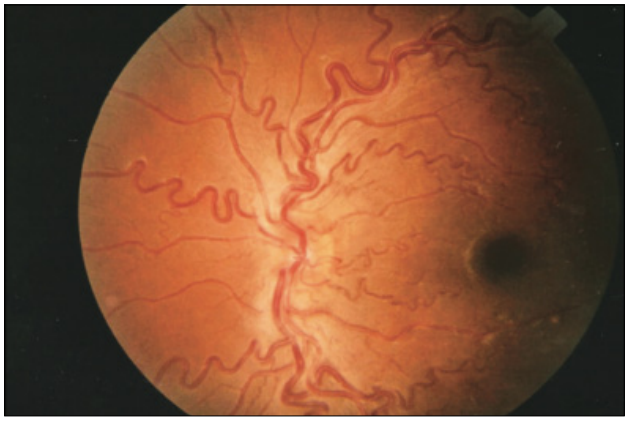

FIGURE 1 Increased vascular tortuosity.[1]

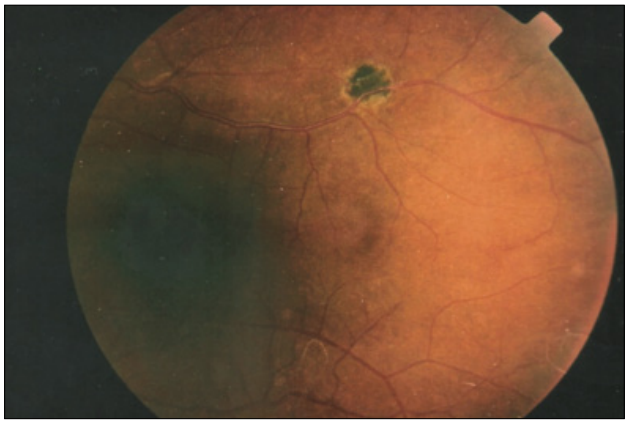

FIGURE 2 "Black sunburst."[1]

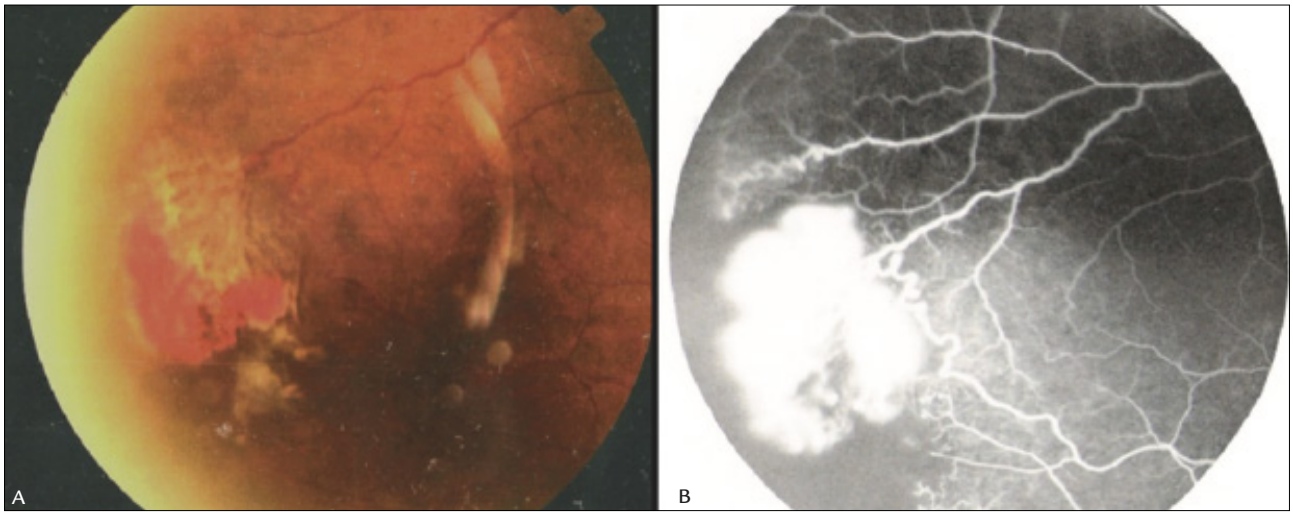

FIGURE 3 A. "Sea fan." B. Angiographic appearance of the "sea fan."[1]

The latter is widely used to treat the typical stage III lesion (sea fan) of proliferative retinopathy. However, patients in stages I and II are not treated, since the treatment of ischemic lesions in these stages does not prevent the formation of sea fan (Figure 3), and most patients do not develop the complication.[8,22] There is also surgical treatment, which is indicated for complications of proliferative retinopathy, such as retinal detachment and vitreous hemorrhage.[8,23]

According to Clarkson,[24] 1992, prophylactic photocoagulation may play a role in the treatment of selected patients with SC proliferative sickle cell retinopathy, but none of the studies reported to date have demonstrated that this treatment improves long-term visual outcome compared to natural progression, as documented in the present study. The similar visual results in the eyes analyzed in our study during the natural progression of the disease compared to those treated with photocoagulation should not be unexpected, because there is a greater predilection for spontaneous involution or neovascular tissue infarction in SC disease as opposed to neovascularization that develops in other vascular diseases of the retina. Clarkson[24] suggests that a multicenter controlled clinical trial designed to study eyes at greater risk should be considered. There is, however, no clear definition of the risk factors leading to these advanced stages, and the value of treatment is uncertain.[24]

In addition, since ocular disorders of retinopathy result from a systemic pathological process, prevention can be done with appropriate treatment of anemia using several emerging approaches, such as:[8,25] increase in fetal hemoglobin using hydroxyurea, omega-3 and erythropoietin, 2-deoxy-5-azacytidine; erythrocyte hydration (clotrimazole, magnesium pidolate); anti-inflammatory and anti-adhesive drugs (anti-adhesive antibodies, anti-integrin antibodies, anti-Willebrand factor, sulfasalazine, statins); antioxidant therapy (glutamine, deferiprone); antithrombotic agents (heparin, ticlopidine, warfarin); vasodilatation (nitric oxide, arginine, Flocor); decrease in hemoglobin S by transfusion and apheresis; transplantation of hematopoietic cells and gene therapy.

Conclusion

Considering that sickle cell retinopathy is a complication that causes 42% of blindness in the affected patients, we point out the importance of new studies on the subject, since there is a gap especially in randomized clinical trials. The importance of our review is to draw attention to the need for periodic ophthalmologic monitoring in patients with anemia since childhood, aiming at prevention, diagnosis and early treatment of the disease.

Resumo

Retinopatia da doença falciforme: revisão da literatura

As hemoglobinopatias são um grupo de doenças hereditárias que causam alterações quantitativas ou qualitativas no formato, na função ou na síntese de hemoglobinas. Uma das mais comuns é a anemia falciforme, cuja patogenia é a foicização das hemácias, causando fenômenos vaso-oclusivos. Dentre as manifestações oculares possíveis, a mais representativa é a retinopatia, que pode levar à cegueira caso não seja tratada. Por isso, é importante que haja o acompanhamento oftalmológico perió-

dico desses pacientes, a fim de obter diagnóstico precoce e abordagem terapêutica adequada. Esta última pode ser de maneira direta, com tratamento das lesões oculares, ou de forma sistêmica.

Palavras-chave: doenças retinianas, anemia falciforme, revisão, hemoglobinopatias.

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ERRATUM

http://dx.doi.org/10.1590/1806-9282.63.12.1104

In the article "Common mental disorders in medical students: A repeated cross-sectional study over six years", DOI: http://dx.doi.org/10.1590/1806-9282.63.09.771, published in the journal Rev Assoc Med Bras, 63(9):771-778, on page 771, where it reads:

"We performed logistic regression and correspondence analysis."

Change to:

"We performed *Poisson* regression and correspondence analysis."

On page 777, where it reads:

"Realizadas regressão logística e análise de correspondência."

Change to:

"Realizadas regressão de *Poisson* e análise de correspondência."

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