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SUMMARY

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SECTIONS

ORIGINAL ARTICLES

REVIEW ARTICLES

Cardiovascular disease and yellow fever

Doença cardiovascular e febre amarela

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All of us cardiologists have been asked about vaccination against yellow fever during consultations, as well as in phone calls or messages from patients or family members. The reason is that the outbreak of the disease has appeared on the news daily causing the whole country to worry.

Even in the absence of a consensus on universal vaccination, the current epidemiological situation requires the intensification of measures in the short term due to the risk of triggering a catastrophic urban epidemic with high morbidity and mortality.

One of the most difficult decisions involving medical liability is in the recommendation for patients over the age of 60 years, when there is a higher incidence of adverse events that, although rare, can be serious and fatal.

These complications have been reported in studies with the non-fractional vaccine on a regular basis and we do not know if they would be even rarer with the fractional vaccine that is now being used.

It must be understood that this situation is dynamic, like any epidemic or pandemic outbreak, and there is a permanent need for reassessment, clarification and guidance. Thus, public health authorities and medical societies have advised that patients over 60 years should consult their professional for advice, which requires careful evaluation of the epidemiological risk. It is physician's responsibility to define the protection of this age group in the population. If they are not vaccinated and become infected, they can rapidly progress to severe forms that will increase the complications of any underlying conditions. The decision involves comparing the risk of developing complications, estimated for the elderly (with the entire vaccine) at 1 per 100,000 (60 to 70 years-old) or 1 per 30,000 (> 70 years-old) versus 1-2 per 1 million in non-elderly individuals.

Therefore, the Ministry of Health published a generic guide:

Ministry of Health – Guide to vaccination criteria against yellow fever

Precautions

Thorough and individualized risk and benefit assessment for vaccine recommendation is required as follows:

- Acute moderate or severe febrile conditions.
- First yellow fever vaccination in people aged 60 and over.
- Blood or organ donors.
- HIV-infected individuals, asymptomatic and with moderate immunosuppression according to CD4 count.
- People with potentially autoimmune diseases.
- People with hematological diseases.
- Patients who have developed demyelinating neurological disease within 6 weeks after the previous dose of the vaccine.
- Pregnant and breastfeeding women.

Contraindications

- Children under 6 months of age.
- Individuals with a history of severe adverse events with previous doses.
- Those with a history of laboratory-proven anaphylaxis with previous doses or anaphylaxis related to the substances present in the vaccine (chicken egg and its derivatives, bovine gelatine or others).
- Patients with severe immunosuppression of any nature.
- Patients undergoing organ transplantation.
- Patients with previous history of thymus disease.
- Patients with systemic lupus erythematosus.

However, some special situations in cardiological patients can cause doubt.

We list some of them below, which can make it easier for us to guide patients:

1. I am over 60 years and am going to travel to an area with a high incidence of yellow fever; should I be vaccinated?
If you are over 60 years old, but do not meet the contraindication criteria, vaccination is recommended, even if you have a chronic disease such as heart failure (HF), diabetes mellitus (DM) or coronary artery disease (CAD). If you have any of the contraindications, you should not travel or, if it is unavoidable, use insect repellent and avoid exposing yourself.

2. I am over 60 years, I live in an urban area with a low incidence of the disease and I will not travel to any endemic area, should I be vaccinated?
In this case, the risk of vaccination is greater than the benefit.

3. Can a patient aged less than 60 years but with a chronic disease (such as HF, DM or CAD) be vaccinated?
Yes, and should be. The simple presence of chronic disease does not contraindicate vaccination.

4. My patient is young and has a complex congenital heart disease. Can he be vaccinated?
Yes, he can. Patients with congenital heart diseases, even complex ones, and aged over 6 months can be vaccinated.

5. My patient has valvulopathy with a history of rheumatic fever (which is ultimately an autoimmune disease) but has not had activity. Should he be vaccinated?
There is nothing specific for rheumatic fever and all patients with controlled autoimmune diseases, in the absence of immunosuppression, may theoretically receive the vaccine except for lupus, which is a contraindication. In this case, there are still controversies.

6. Does vaccination against yellow fever cause myocarditis?
Yellow fever virus infection is one of the causes of myocarditis; post-vaccinal myocarditis alone is possible but extremely rare.

7. My patient had myocarditis with no defined etiology. Can he be vaccinated?
Yes, he can. Vaccination is not contraindicated, whether the ventricular function has been recovered or not.

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Contralateral prophylactic mastectomy

Mastectomia profilática contralateral

Authorship: Brazilian Medical Association

Participants: Ricardo Santos Simões[1], Wanderley Marques Bernardo[1], Antonio Silvinato[1], Thais A. Frank[1], Renata Buzzini[1]

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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 patient, i.e. women diagnosed with unilateral breast cancer; I for intervention, i.e. simple or total mastectomy, skin-sparing mastectomy, and nipple-areola complex-sparing mastectomy; C for comparison with women who did not undergo contralateral prophylactic mastectomy, and O for the outcome of reduction of the incidence of breast cancer in the contralateral breast.

Based on the structured question, we identified the descriptors that formed the basis of the search for evidence in the databases: Medline-Pubmed and Cochrane. A total of 424 studies were retrieved, of which five were selected to answer the clinical questions (Annex I).

Clinical question

Is contralateral prophylactic mastectomy (CPM) in women with unilateral breast cancer associated with a decline in the incidence of breast cancer in the contralateral breast?

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

This guideline is intended for physicians and medical students and aims to assess whether contralateral prophylactic mastectomy (CPM) in women with unilateral breast cancer is associated with a decline in the incidence of breast cancer in the contralateral breast.

Introduction

Breast cancer is the neoplastic disease that most affects women in Brazil and the world. In Brazil, for the year 2016, 57,960 new cases were expected.[1]

Women with a history of breast cancer are at increased risk for developing contralateral breast cancer, and this risk is related to a variety of factors, including genetics, family history and characteristics of the primary cancer itself.[2,3]

Although studies have shown the efficacy of adjuvant endocrine therapy to reduce the risk of contralateral breast cancer, a growing proportion of women in the early stages of breast cancer have undergone surgical removal of the non-affected breast through risk reducing mastectomy of the contralateral breast.[4,5] Despite the substantial benefits associated with reducing the risk of breast cancer itself, its risk-benefit ratio is controversial because of the negative impact of surgery on self-image, sexuality and quality of

life, in addition to complications related to the procedure itself.[6] Thus, in order to support decision-making by the indication or not of the contralateral prophylactic mastectomy using robust evidence, a systematic review was carried out to evaluate whether CPM in women with unilateral breast cancer is associated with a decline in the incidence of breast cancer in the unaffected breast.

Data extraction

Data referring to a total of 5,532 patients were analyzed, with 2,700 of these women undergoing contralateral prophylactic mastectomy following a personal history of unilateral breast cancer. The mean age of these patients was 46 (Table 1). With mean follow-up time ranging from 3.5 to 17.3 years, it was observed that the contralateral prophylactic mastectomy was associated with a reduction in the incidence of breast cancer in the contralateral breast with values ranging from 78 to 98% and overall risk reduction of 95% (RR=0.05; 95CI 0.02-0.11) (Table 2).

Regarding data on overall survival, studies have conflicting results, some with increased survival[7] and others not confirming this gain.[8-10] Two studies analyzed the incidence of distant metastasis, with a difference between women treated and not treated with CPM (RR=0.65; 95CI 0.46-0.91) (Table 3).[7,11] (**B**).

Studies have demonstrated that contralateral prophylactic mastectomy is associated with a decline in the incidence of contralateral breast cancer in approximately 95% of women with a personal history of unilateral breast cancer.[12,13] (**B**) Supporting these findings, there was a 95% reduction in the incidence of breast cancer; however, the impact on overall survival or even breast cancer-free survival is uncertain, as evidenced in another systematic review that included observational studies.[13] (**B**)

In a retrospective study that showed an average follow-up of around 17 years, the authors reported a 94% lower incidence of contralateral breast cancer in women with stage I or II breast cancer who had undergone therapeutic mastectomy combined with contralateral prophylactic mastectomy.[7] (**B**) The study, with significant long-term follow-up, showed that contralateral prophylactic mastectomy was also associated with superior overall survival and disease-free survival outcomes, although a difference with respect to the incidence of distant metastases was not found.[7] (**B**) On the other hand, other authors, analyzing women with mutations in BRCA1 and BRCA2 genes previously treated for unilateral invasive breast cancer (stage I–IIIa), did not find an increase in overall survival after adjustment for bilateral prophylactic oophorectomy.[9] (**B**) These authors, with no adjustment for prophylactic oophorectomy, found greater overall survival at 5 years in patients undergoing contralateral prophylactic mastectomy, but attributed these findings to the higher mortality observed in the group of patients kept under surveillance.[9] (**B**)

Supporting these findings, in another retrospective study in which more than 1,000 patients with breast cancer were analyzed, the contralateral prophylactic mastectomy was not associated with greater overall survival.[10] (**B**) With a mean follow-up of 6.8 years, the authors found greater disease-free survival for patients undergoing contralateral prophylactic mastectomy (55% versus 28%,

TABLE 1 Studies selected.

Authors and publication year	Type of study	Age	N/CPM	N/Control	Follow-up time	Endpoints
Peralta et al., 2000	Retrospective cohort	45.5	0/64	36/182	6.2 years for CPM and 6.8 years for the control group	Overall survival, disease-free survival, incidence of cancer in the contralateral breast
van Sprundel et al., 2005	Retrospective cohort	CPM=41.5±0.9 Control=46.7±1.1	1/79	6/69	3.5 years	Overall survival, incidence of cancer in the contralateral breast
Boughey et al., 2010	Retrospective cohort	NA	2/385	31/385	17.3 years	Overall survival, disease-free survival, incidence of cancer in the contralateral breast, distant metastases
Herrinton et al., 2005	Retrospective cohort	CPM=50 Control=58	5/1,072	69/317	5.7 years	Cancer incidence in the contralateral breast; overall survival
King et al., 2011	Retrospective cohort	CPM=44.8 Control=53.2	0/407	14/2,572	4.4 years for CPM and 6.8 years for the control group	Incidence of contralateral breast cancer, distant metastases

Age: years ± standard deviation; N: patients with breast cancer; CPM: contralateral prophylactic mastectomy; Control: not treated with CPM; Follow-up time: years; NA: not available.

TABLE 2 Incidence of breast cancer in the contralateral breast.

Study or subgroup	CPM Events	CPM Total	Control Events	Control Total	Weight	Risk ratio M-H, Random, 95CI
Boughey et al., 2010	2	385	31	385	25.3%	0.06 [0.02, 0.27]
Herrinton et al., 2005	5	1,072	69	317	44.1%	0.02 [0.01, 0.05]
King et al., 2011	0	407	14	2,572	8.2%	0.22 [0.01, 3.64]
Peralta et al., 2000	0	64	36	182	8.5%	0.04 [0.00, 0.62]
van Sprundel et al., 2005	1	79	6	69	13.9%	0.15 [0.02, 1.18]
Total (95CI)		**2007**		**3,525**	**100.0%**	**0.05 [0.02, 0.11]**
Total events	8		156			

Heterogeneity: $Tau^2 = 0.22$; $Chi^2 = 5.15$, df = 4 (p=0.27); $I^2 = 22\%$

Test for overall effect: Z = 7.04 (p<0.00001)

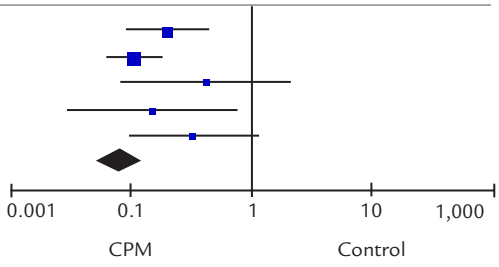
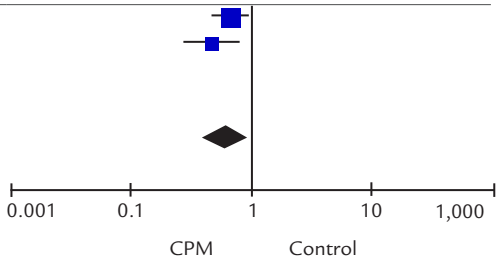


TABLE 3 Incidence of distant metastases.

Study or subgroup	CPM Events	CPM Total	Control Events	Control Total	Weight	Risk ratio M-H, Random, 95CI
Boughey et al., 2010	60	385	82	385	66.2%	0.73 [0.54, 0.99]
King et al., 2011	15	407	187	2,572	33.8%	0.51 [0.30, 0.85]
Total (95CI)		**792**		**2,957**	**100.0%**	**0.65 [0.46, 0.91]**
Total events	75		269			

Heterogeneity: $Tau^2 = 0.22$; $Chi^2 = 1.51$, df = 1 (p=0.22); $I^2 = 34\%$

Test for overall effect: Z = 2.46 (p=0.01)



p=0.01), but did not identify a difference for the rate of overall survival (64% versus 48%, p=0.2).[8] (**B**) Even after adjusting the groups for prognostic factors, they did not find an improvement in the overall survival rate after 15 years of follow-up.[8] (**B**)

The lack of translation to benefit of contralateral breast cancer control, in terms of greater overall survival and disease-free survival, based on a decline in the incidence of breast cancer with the indication of CPM, is not unusual. For many women with early-stage breast cancer, the risk of metastatic disease is greater than that for contralateral breast cancer.[2] (**B**) Therefore, it is possible that the benefits of CPM in terms of disease-free survival are observed only in certain patient subgroups. In fact, another study based on the SEER (Surveillance, Epidemiology and End Results) database showed that, in patients with estrogen receptor-positive breast cancer, the contralateral prophylactic mastectomy was not associated with higher specific survival related to breast cancer.[14] (**B**)

Recommendation

For women who have already been diagnosed with unilateral breast cancer, the contralateral prophylactic mastectomy reduces the incidence of breast cancer in the contralateral breast and distant metastases. With respect to survival (overall or disease-free), the evidence is limited.

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Conflict of interest

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

Annex I

Structured question

- **P** – Women diagnosed with unilateral breast cancer.
- **I** – Simple or total mastectomy, skin-sparing mastectomy, and nipple-areola complex-sparing mastectomy.
- **C** – Women who did not undergo contralateral prophylactic mastectomy.
- **O** – Reduction of the incidence of breast cancer in the contralateral breast.

Search strategy

- **PubMed-Medline:** (Prophylactic Mastectomy OR Prophylactic Mastectomies OR (Mastectomy AND prevention and control)) AND contralateral.
- **Cochrane:** Prophylactic Mastectomy AND contralateral.

Study selection

Initially selected by the title, then by the abstract, and finally by their full text, the latter being subject to critical evaluation and extraction of results related to the endpoints (Table 1).

Retrieval of relevant articles was conducted through the strategy described in Chart 1 using as primary databases Medline and Cochrane for search completed in April 2017, without restricting the year of publication or language. The process of retrieving articles, as well as evaluating the titles and abstracts obtained, was conducted by two researchers qualified to conduct systematic reviews (W.M.B. and R.S.S.) independently and blindly, following the criteria of inclusion and exclusion according to the PICO components.[15] Then, the selected articles were critically evaluated to decide whether they would be included in the review. Whenever there was disagreement over the selection of studies among the investigators, a third reviewer was consulted (A.S.). To analyze the methodological quality of the included articles, a Cochrane Collaboration tool was used, excluding three domains related to the evaluation of randomized clinical trials (adequate generation of random sequence, concealment of allocation and blinding), not applicable to this review.[16]

Language

We included studies available in Portuguese, English or Spanish.

According to publication

Only full-text studies were considered for critical assessment.

Critical evaluation and strength of evidence

The strength of the evidence from experimental studies was defined taking into account the study design and corresponding bias risks, the results of the analysis (magnitude and precision), relevance and applicability (Oxford).[17]

Articles retrieved

The process of searching, identifying and selecting articles is demonstrated in Figure 1. From the elaborated search strategies, 424 articles were retrieved, of which 32 were selected after reading the title and abstract. Of these studies, five were selected for inclusion in the systematic review and meta-analysis. No randomized clinical trials were retrieved. The main reason for excluding articles was the fact that they were not related to the PICO components. The methodological evaluation of included studies

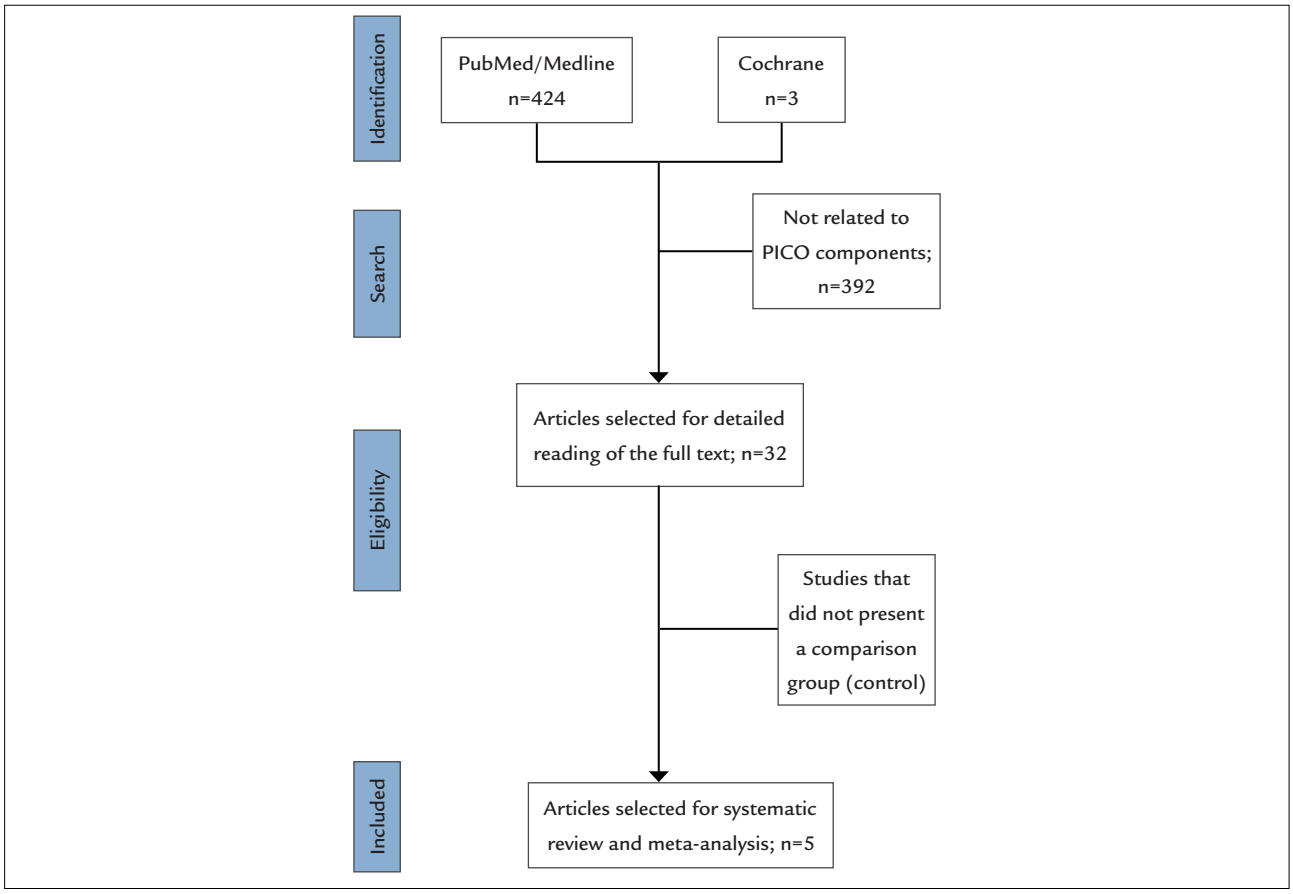

FIGURE 1 Study selection flowchart.

according to the Cochrane Collaboration tool is presented in Figures 2 and 3. Figure 4 shows the evaluation of publication bias using a Funnel plot.

Inclusion and exclusion criteria

In the selection of studies, we included only those that analyzed women diagnosed with unilateral breast cancer and who were subjected to contralateral prophylactic mastectomy, which were compared to a control group comprising women who were not treated with contralateral prophylactic mastectomy, continued to be monitored. The procedures related to prophylactic or reductive mastectomy of the contralateral breast were: simple or total mastectomy, skin-sparing mastectomy, and nipple-areola complex-sparing mastectomy, performed in the breast without clinical or radiological evidence of the presence of malignancy.

Critical appraisal method

Whenever, after applying the inclusion and exclusion criteria, the selected evidence was defined, an appropriate Critical Assessment Checklist was applied.

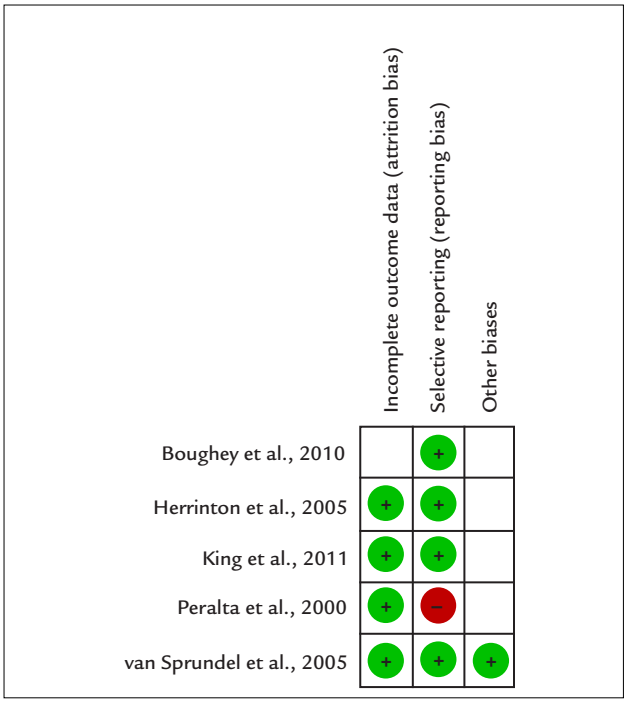

FIGURE 2 Risk of bias, author's judgment and criteria used to judge.

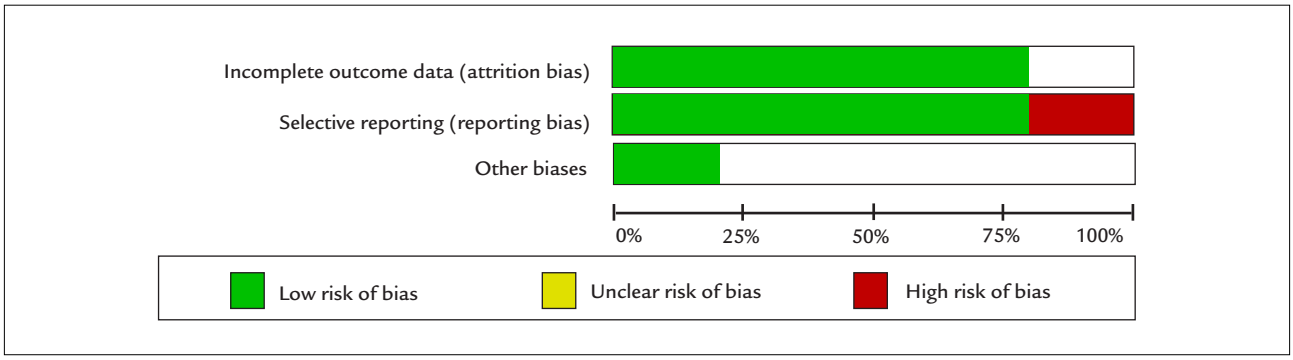

FIGURE 3 Bias risk graph expressed in percentages.

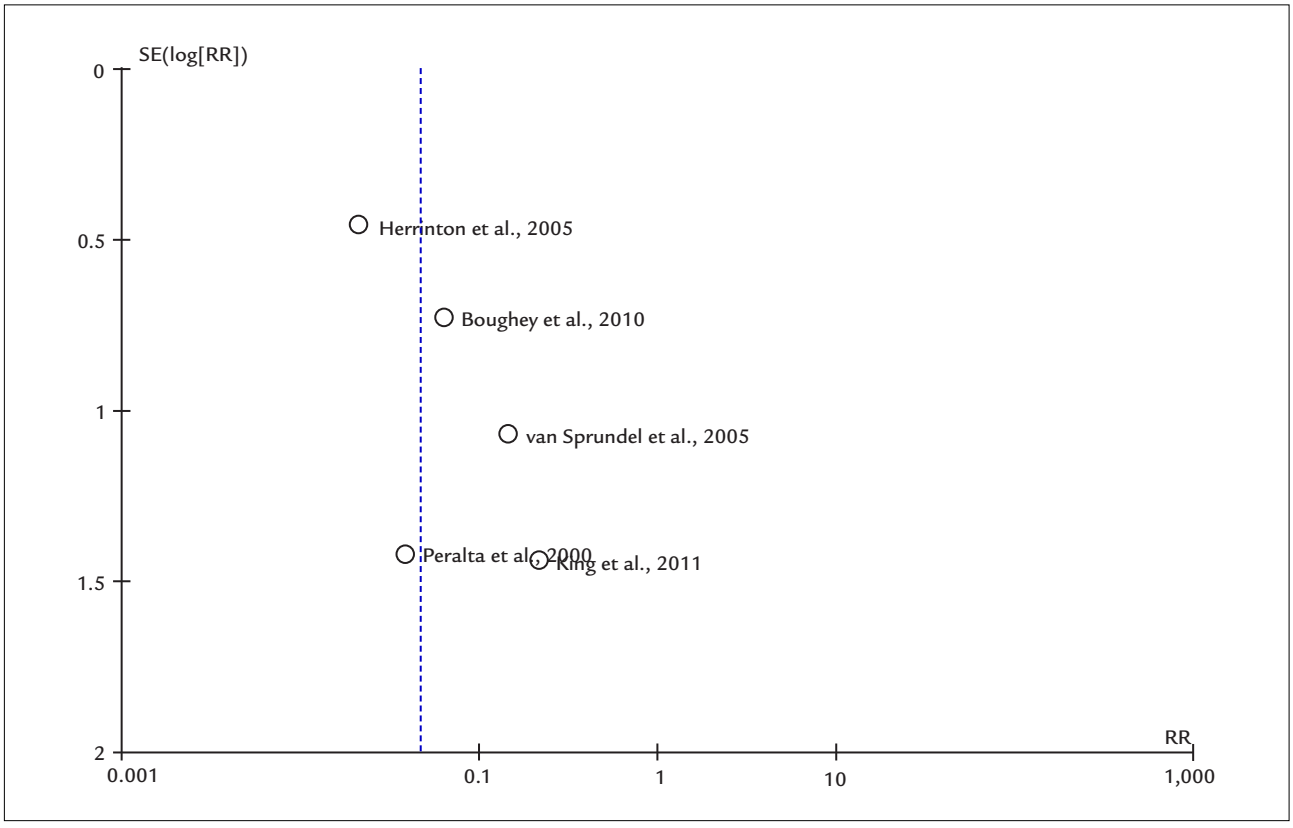

FIGURE 4 Evaluation of publication bias.

Exposure of results

The information obtained from the studies selected for the systematic review was inserted in a table where the following characteristics were described if present in the articles: author's name and year of publication, study design, number of patients who developed breast cancer, number of patients treated or not treated with contralateral prophylactic mastectomy, age, follow-up time and endpoints analyzed (Table 1).

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.

Nihil novi sub sole

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Dear Editor,

In their brilliant review, Fernandes and Barreto discussed the association between physical activity and vitamin D.[1] They gave some interesting historical information. They also wrote that researchers "indicating the practice of physical activity in outdoor settings (...) do not mention the importance of vitamin D" and in another study "do not mention physical activity with the sun exposure." We could not agree with this. At the beginning of the 19th century, Jędrzej Śniadecki, a professor of pharmacy and chairman of medicine in Vilno, capital of Lithuania, published a work entitled "On the Physical Upbringing of Children." He presented the methods of child upbringing to shape their bodies properly, taking care of both the physical and mental development. In the second edition of this work, in 1822, he proposed for the first time sunbathing as a method of rickets treatment. It later appeared as treatment with vitamin D.[2,3] Śniadecki was a professor of chemistry but also a family doctor, and, working in the northern part of Europe, he knew very well how harmful the lack of the sunlight could be.

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Statistical comments on "Antiretroviral changes during the first year of therapy"

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Dear editor,

We read with great interest a recent article by Bandeira et al.[1] entitled "Antiretroviral changes during the first year of therapy." In this study, the authors investigated the first year of highly active antiretroviral therapy (HAART) in patients from a reference center on HIV/AIDS management in Fortaleza. They evaluated CD4 T lymphocyte (LTCD4) count and viral load (VL) over the course of the treatment (4 time-points of measurement including before treatment, 2-4 months, 5-8 months and 9-12 months). As stated in the methods section and chart 1 of the article, the authors used Student t-test (independent t-test) and Mann-Whitney test for comparison of LTCD4 count and VL between different time-points of measurement. Indeed, the authors investigated levels of the numerical variables in one sample of the patients in different times and assumed them as independent measurements. Since they used only one sample of the patients and measured numerical variables in them during different time-points, their measurement are dependent. Student t-test and Mann-Whitney test are used to compare the differences between the means of two independent (unrelated) groups, but the measurements of the mentioned study are not independent.[2-6] Therefore, after assessment of the normal distribution of the variables, due to dependence of measurements, they must use dependent t-test (paired t test) or Wilcoxon signed rank test for comparisons between different time-points of measurement.[4,7-10]

Taken together, we believe that most of the statistical tests used in this study (including Student t-test and Mann-Whitney test) are inappropriate, and the authors' valuable study could be better used as citable experimental evidence if analyzed with appropriate statistical tests (including dependent t-test or Wilcoxon signed rank test).

Conflict of interest

The authors declare that they have no conflict of interest.

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Microcephaly caused by congenital Zika virus infection and viral detection in maternal urine during pregnancy

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Summary

Currently Latin America is undergoing a major epidemic of Zika virus, which is transmitted by *Aedes* mosquitoes. Concern for Zika virus infection has been increasing as it is suspected of causing brain defects in newborns such as microcephaly and, more recently, potential neurological and autoimmune complications including Guillian-Barré syndrome and acute disseminated encephalomyelitis. We describe a case of virus infection in a 25-year-old woman during the first trimester of her pregnancy, confirmed by laboratory tests only for the detection of viral particles in maternal urine, with imaging studies demonstrating the progression of cranial and encephalic changes in the fetus and later in the newborn, such as head circumference reduction, cerebral calcifications and ventriculomegaly.

Keywords: Zika. Virus. Pregnancy. Urine. Microcephaly.

Case report

Female patient, 25 years old, pregnant with gestational age of 19 weeks and 4 days as per date of the last menstrual period, referred for obstetric morphological ultrasound evaluation. She reported that, between 9 and 11 weeks of gestation, she had episodes of mild fever accompanied by small reddish skin patches and mild pruritus for a week. The patient denied having comorbidities and exposure to teratogenic drugs or substances. Infection with toxoplasmosis, rubella, cytomegalovirus, herpes simplex and syphilis were ruled out based on serum serology. At the end of the symptomatic period, Zika virus (ZIKV) detection was performed using RT-PCR in the patient's urine, yielding a positive result. Maternal serology for ZIKV infection was inconclusive.

Ultrasonographic examination (Figure 1) showed enlarged lateral ventricles, measuring 1.4 cm on the left and 1.6 cm on the right, with a biparietal diameter and cephalic perimeter within the expected range, measuring 4.8 cm and 17.8 cm, respectively, and no other perceptible abnormalities. Gestational age based on biometry was compatible with 21 weeks and 2 days.

Two weeks later, on fetal magnetic resonance imaging (Figure 2), there was a marked lateral ventriculomegaly on both sides associated with significant volume reduction and diffuse thinning of the cerebral parenchyma, with cranial circumference measuring about 19.9 cm.

After 10 weeks, obstetrical ultrasound indicated a fetus with a general measurement compatible with 33 weeks and 3 days, presenting bilaterally enlarged lateral ventricles, biparietal diameter and cranial circumference below the 5th percentile of normality, measuring 6.5 cm and 22.9 cm, respectively.

Delivery was by cesarean section 6 weeks later. Transfontanelle ultrasonography was performed in the newborn, which demonstrated markedly dilated lateral ventricles, cerebral parenchyma with signs of relevant volume reduction, extensive periventricular calcifications and simplification of the cerebral convolutions (lissencephaly). These findings were confirmed by computerized tomography (Figure 3) on the same day. Serology for Zika virus was not investigated in the newborn.

Discussion

Latin America is currently undergoing a major epidemic of ZIKV, an arbovirus of the Flaviviridae family and genus Flavivirus transmitted by *Aedes* mosquitoes, including

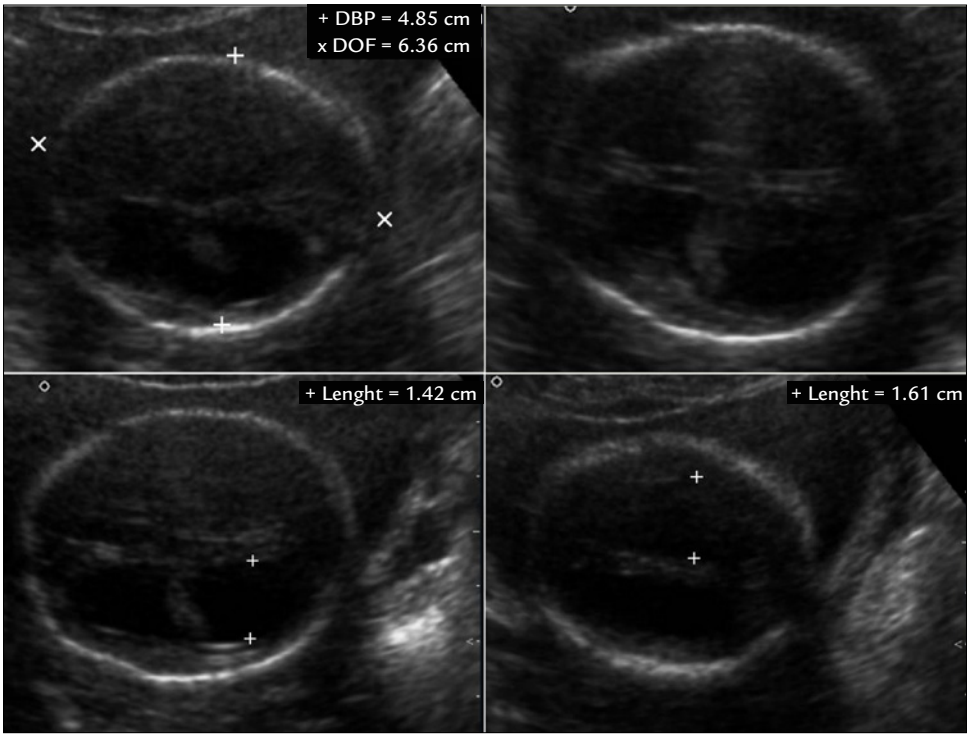

FIGURE 1 Obstetric ultrasonography showing the fetal cephalic pole with enlarged lateral ventricles, and biparietal and occipitofrontal diameters within the expected range.

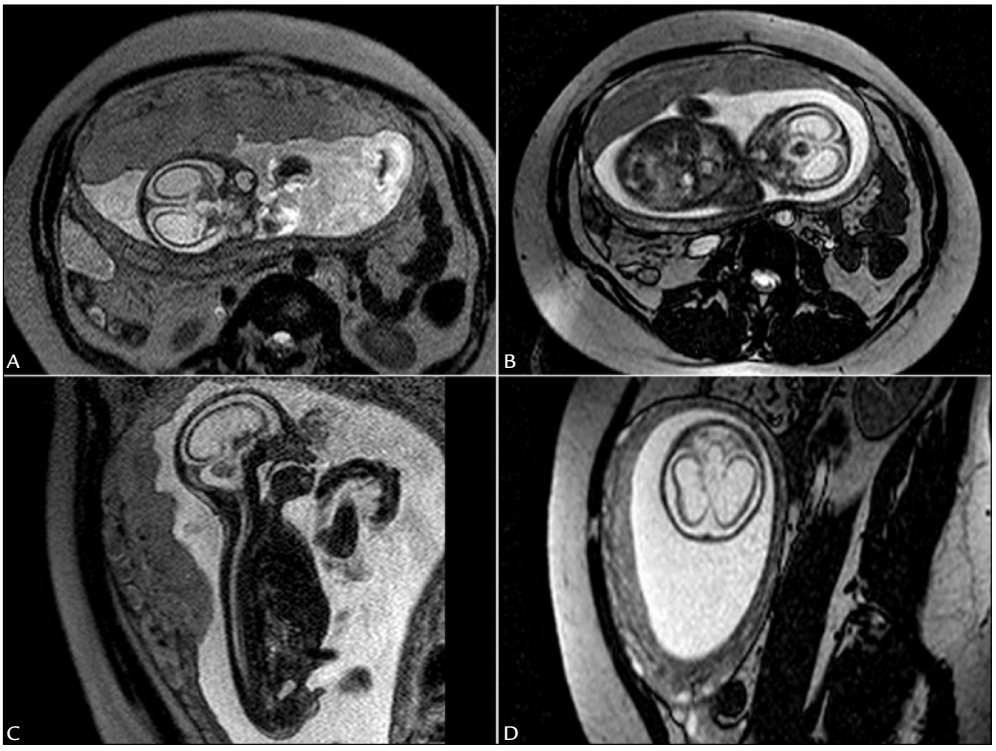

FIGURE 2 Fetal magnetic resonance imaging, T2-weighted sequence demonstrating pronounced lateral ventriculomegaly on both sides associated with significant volumetric reduction and diffuse thinning of the cerebral parenchyma.

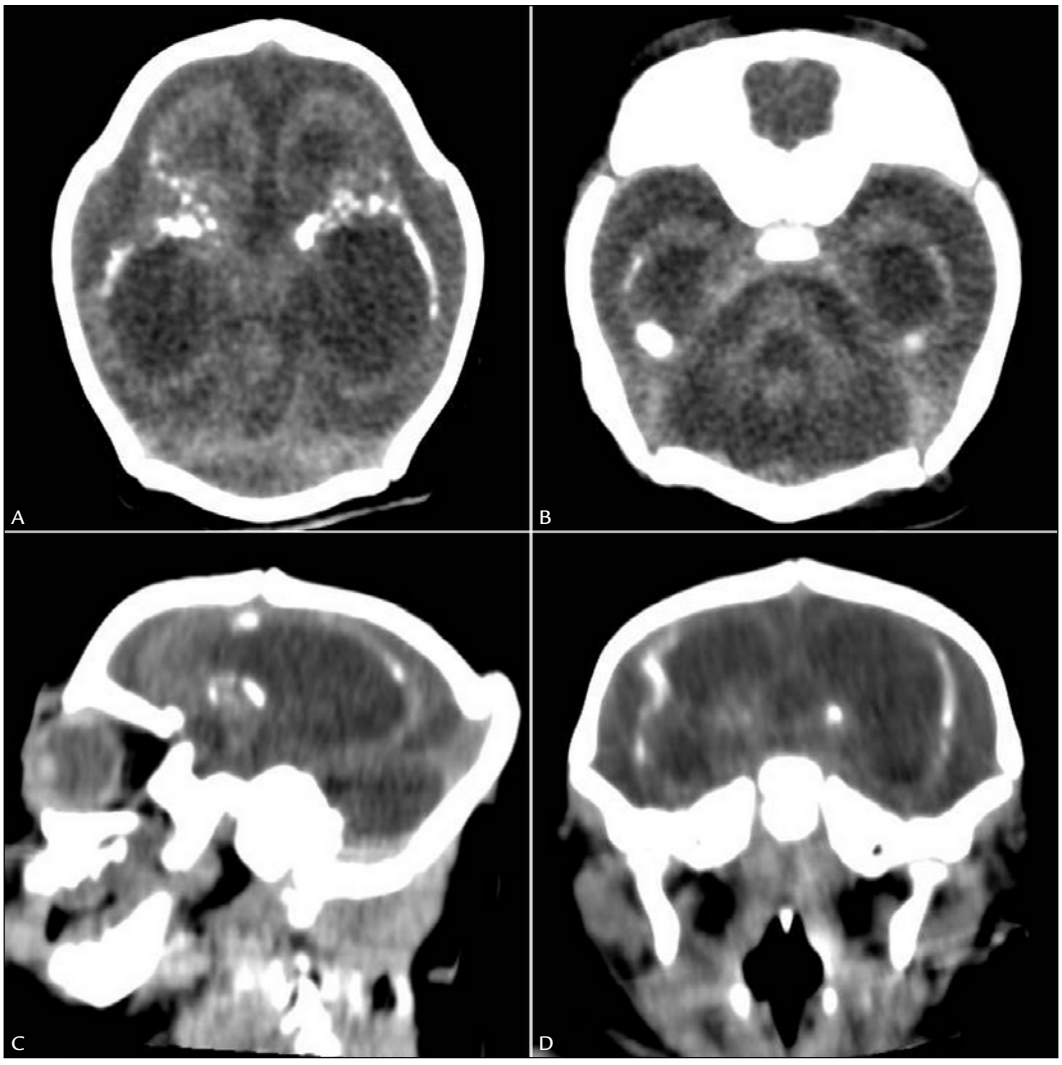

FIGURE 3 Computed tomography of the newborn characterizes markedly dilated lateral ventricles, cerebral parenchyma with signs of marked volumetric reduction, extensive periventricular calcifications and simplification of the cerebral convolutions (lissencephaly).

Aedes aegypti. The name derives from the Zika forest in Uganda, which is where the virus was first isolated in 1947.[1] Concern for ZIKV infection is increasing, as it is suspected of causing brain defects in newborns, such as microcephaly and, more recently, potential neurological and autoimmune complications such as Guillain-Barré syndrome and acute disseminated encephalomyelitis (ADEM).[2] According to the World Health Organization (WHO), microcephaly consists of a malformation in which the head circumference is two or more standard deviations smaller than expected for a given gestational age and gender of the newborn.[3]

As of May 2015, several cases of ZIKV infection have been reported in Brazil associated with an increasing number of neonates with congenital microcephaly born to women infected during pregnancy.[4] Brazil is the most affected country, with a preliminary estimate of about 440,000 to 1.3 million autochthonous cases by December 2015.[5]

The infection tends to be benign, with estimated duration of symptoms from 2 to 7 days and include maculopapular rash, fever, arthralgia and conjunctivitis. Myalgia, headache, retro-orbital pain, edema, vomiting, pruritus, dizziness, mucous ulcers, diarrhea and constipation may occur as well.[6]

Diagnosis can be tricky based on clinical signs and symptoms alone, which may overlap with other endemic arboviruses in similar areas.[2] A definitive diagnosis depends on virus isolation or positive polymerase chain reaction (PCR). Serologic testing with IgM antibodies detected by immunoenzymatic (ELISA) or immunofluorescence assay can be performed to diagnose the acute infection.[3] Identification of ZIKV RNA in amniotic fluid

in addition to findings of congenital microcephaly assessed on prenatal ultrasonography was reported in two cases of pregnant women. This led to concern about the possible association between congenital microcephaly and the recent outbreak of this infection in Brazil.[4]

RNA detection in urine can occur for longer than the detection in serum after the onset of infection. There are also reports of ZIKV detection in the saliva of neonates and adults.[2] In a study, Kirsten et al. found a percentage of 62% of patients evaluated with serum serology negative for ZIKV, but with viral positivity detected in urine, which is meaningful since our case report describes a similar situation.[7]

The radiological findings range from calcifications located predominantly at the junction between the cortical region and the subcortical areas of white matter, malformations of cortical development and sulcation, microcephaly with cortical gyri that present normal thickness associated with areas of thick cortex (polymicrogyria) predominantly located in the frontal lobes, ventriculomegaly with a predominant increase of the posterior portions of the lateral ventricles, abnormalities of the corpus callosum (hypogenesis and hypoplasia) and delayed myelination.[8]

Notably, maternal infection, even if subclinical, can lead to severe congenital abnormalities, and evaluation with serial imaging may demonstrate the progression of the findings. Ultrasonographic findings in prenatal care consist of decreased head circumference and, rarely, increased cranial circumference. The vast majority of newborns will present with more severe calcifications occurring more frequently at the junction of white-gray matter compared to what is usually seen in TORCH infections (toxoplasmosis, rubella, cytomegalovirus and herpes simplex, as well as syphilis, varicella-zoster and parvovirus B1). In the latter, such a location for calcifications is uncommon. Vaccination plays an important role in eradicating fetal disease, but, if immunity is not complete, subsequent exposure to the virus during pregnancy would not prevent infection.[1]

Given that the microcephaly epidemic is a result of congenital Zika virus infection, we recommend that the list of congenital infections referred to as TORCH be renamed to TORCHZ (the letter Z referring to ZIKV).[9] Even though it is possible to conclude that there is no radiographic pathognomic pattern of microcephaly caused by ZIKV, gross calcifications and their cortical-subcortical distribution, also involving the basal ganglia, associated with lissencephaly and ventriculomegaly, in the absence of intracranial hypertension, are characteristic of this type of infection.[1] Advantages of noninvasive collection of urine, saliva, and nasopharyngeal and/or buccal (cheek) samples provide an alternative to diagnose infection in patients whose blood collection may be difficult, including small children, neonates, the elderly, patients with hemorrhagic syndromes and in cases of patient refusal.[2]

Resumo

Microcefalia causada por infecção congênita pelo Zika vírus e detecção viral na urina materna durante a gestação

Atualmente, a América Latina está passando por uma grande epidemia de Zika vírus, transmitido por mosquitos *Aedes*. A preocupação pela infecção pelo Zika vírus vem aumentando, uma vez que é suspeita de causar defeitos cerebrais em recém-nascidos, como a microcefalia e, mais recentemente, potenciais complicações neurológicas e autoimunes, como síndrome de Guillian-Barré e encefalomielite disseminada aguda. Descrevemos um caso de infecção pelo vírus em uma mulher de 25 anos durante o primeiro trimestre de gestação, confirmado dentre os exames laboratoriais apenas pela detecção de partículas virais na urina materna, com estudos de imagens demonstrando a evolução das alterações cranianas e encefálicas no feto e no recém-nascido, como redução do perímetro cefálico, calcificações cerebrais e ventriculomegalia.

Palavras-chave: Zika. Vírus. Gestação. Urina. Microcefalia.

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Endobronchial carcinoid tumor: Radiological findings of a clinical case

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Summary

We describe the case of a female patient, 21 years old, complaining of dyspnea attacks and wheezing 2 years ago. Chest radiography showed volume loss in the left lower lobe and ipsilateral retrocardiac triangular basal opacity. CT scan showed an extensive solid mass with apex protruding into the left main and lower lobar bronchi, causing distal atelectasis. Histopathological and immunohistochemical study of transbronchial biopsy of the lesion revealed a typical carcinoid tumor, confirmed after tumor resection with total left pneumectomy.

Keywords: Carcinoid Tumor. Endobronchial. Pulmonary. Typical. Diagnostic Imaging.

Case report

A female 21-year old patient presenting dyspnea and wheezing attacks for two years. She is overweight, has polycystic ovary syndrome and is currently using oral contraceptives. She does not smoke. The patient reported that three radiographs in the past 6 months revealed consolidation in the left lung base. During this period, treatment regimens for pneumonia were used, without clinical or radiological improvement. On auscultation, rhonchi and wheezing were audible at the base of the left hemithorax. Laboratory tests did not point out any abnormalities.

A new chest X-ray showed loss of volume in the left lower lobe with triangular basal retrocardiac opacity, apex directed towards the ipsilateral hilum and base next to the diaphragmatic dome (Figures 1A and 1B).

Computed tomography (CT) imaging showed a solid expansive mass with apex protruding into the left source bronchus ("tip of the iceberg" sign[1]), causing partial obstruction and distal subsegmentar atelectasis, as well as marked enhancement following intravenous contrast administration (Figures 2A, 2B, 2C and 2D).

After bronchofibroscopy, which confirmed the endobronchial lesion, the anatomopathological and immunohistochemical investigation of the transbronchial biopsy material showed a well-differentiated (typical) carcinoid tumor (Figures 3A, 3B and 3C). A CT scan of the abdomen performed for staging failed to show other neoplastic formations. The therapy selected was left total pneumonectomy, which confirmed the histological finding of tumor in a subsequent investigation.

Discussion

Carcinoid tumors (CATUs) are neuroendocrine neoplasms originating from enterochromaffin cells, commonly found in the gastrointestinal tract. Occurrence in the respiratory tract corresponds to 10-30% of all cases,[1-6] with a bronchopulmonary incidence of 0.22 to 1.57/100,000.[2-4]

They represent up to 2% of lung malignancies,[1,2] often presenting indolent behavior.[1-3,5] Histologically, these tumors are classified as typical (76-90%) or atypical.[1-4] Central bronchial CATUs represent 64-85% of the cases, with a predominance of typical tumors.[1-3]

Typical CATUs affect both sexes,[1-3] with some studies reporting a higher prevalence in women.[2,3] They have two peaks of incidence: one in adolescence and another near the age of 45 years.[1] Metastases occur in 15%.[1]

Symptoms are more frequent in tumors at central sites and some of the main manifestations include recurrent pneumonia, chest pain, cough, hemoptysis and localized wheezing.[1-3,6,7] These tumors can secrete neuroamines and peptide hormones, especially serotonin, growth hormone and adrenocorticotrophic hormone, and may cause carci-

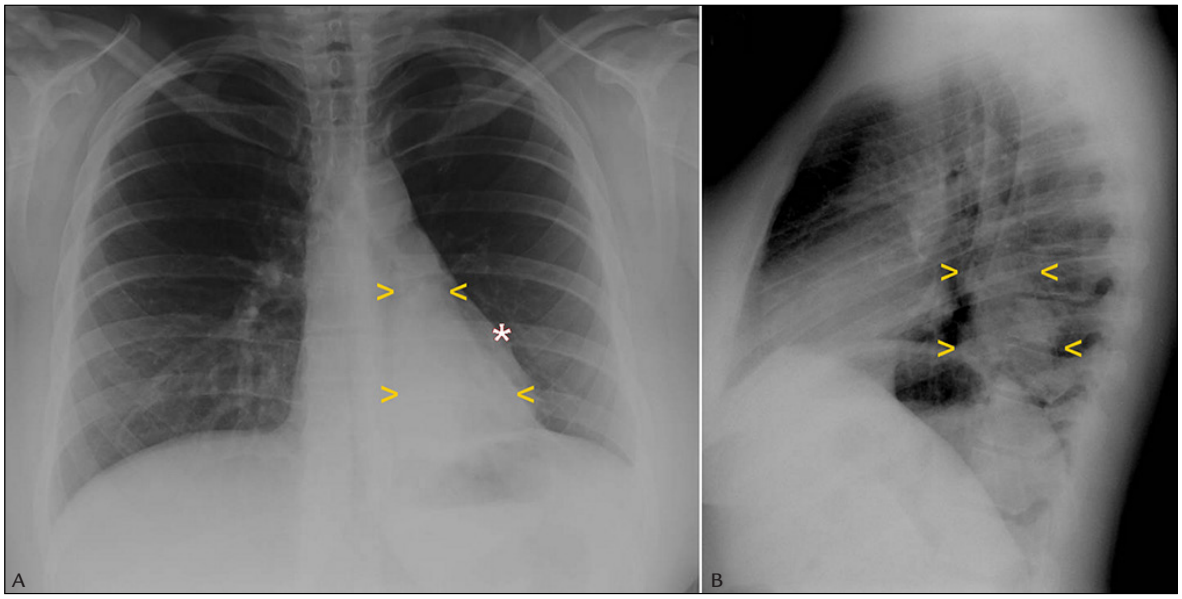

FIGURE 1 Chest radiographs in posteroanterior (A) and lateral (B) view revealing opacity at the left lung base (yellow arrowheads) within the retrocardiac space, appearing predominantly triangular with apex converging to the pulmonary hilum and base blurring of the edges of the diaphragm dome. Note the difference between the edges of the opacity in relation to the cardiac silhouette (*).

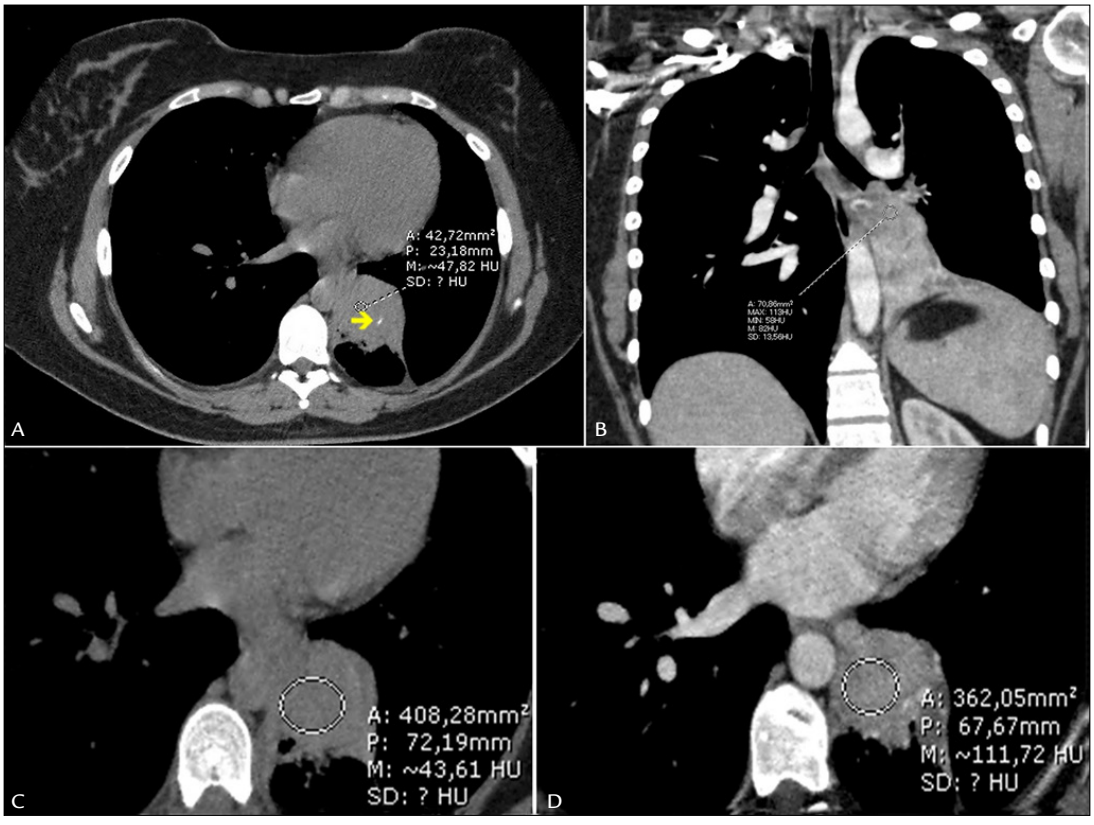

FIGURE 2 Computed tomography scan of the thorax, mediastinal window, without contrast medium (A, C) and after administration of intravenous iodate contrast agent (B, D), revealing a left infrahilar lung mass causing subsegmentar atelectasis of the ipsilateral inferior lobe, with exophytic nodular component protruding into the lumen of the left main and lower subsegmental bronchi. We found scarce punctiform calcifications and diffuse linear foci (A, yellow arrow), as well as accentuated enhancement with contrast medium (B, D) in the extensive mass.

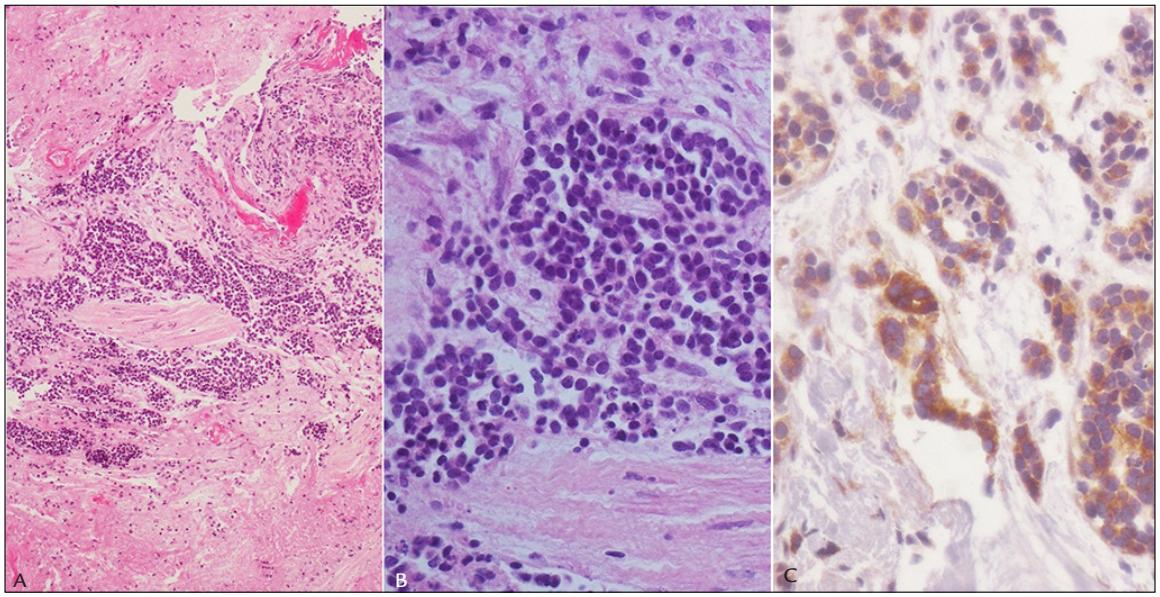

FIGURE 3 A and B. HE staining, showing differentiated small cell epithelial proliferation with rounded, non-pleomorphic nuclei and granular chromatin, with a "salt and pepper" appearance of the tissue. C. Immunohistochemistry with chromogranin A showing cytoplasmic expression of gold-brown DAB in neoplastic cells.

noid syndrome, acromegaly and Cushing's disease (2%), especially in the presence of metastases, with an emphasis on the liver as site of secondary implantation.[1,3]

Radiographs generally reveal well-defined hilar/perihilar masses, rounded or ovoid in shape, associated or not with distal parenchymal disease, such as consolidations or atelectasis. It is rarely possible to distinguish an endoluminal component, with extension to the parenchyma being the most dominant feature.[1-3,7-9]

On CT scan, the presence of two central sites is common (85% in the main, lobar or segmental bronchi), with an appearance of nodule or ovoid mass, well-defined and regular margins, spontaneously hyperdense, associated or not with a distinct endoluminal component or distal parenchymal complications. Punctual or diffuse calcifications are found in 30% of the cases. The stroma in these tumors is typically well-vascularized with homogeneous and intense contrast enhancement.[1-3,7-9]

Despite the primarily endoluminal origin, it is typically observed on CT that most of the mass extends to the adjacent parenchyma, and the endoluminal component may be minimal. This finding is what is called the "tip of the iceberg" sign.[1,8,9]

Prognosis is most often favorable and varies with the typical or atypical character of the lesion, tumor resectability, lymphatic or metastatic dissemination. For staging purposes, the TNM protocol is used for lung neoplasms, and stage I, II and IIIA tumors are considered resectable.[1-3,7-9]

In cases of localized neoplasia, both in the typical and atypical forms, the treatment of choice is tumor resection, with a tendency to preserve as much healthy lung tissue as possible.[2,10,11] Survival rates for the typical and atypical forms 5 and 10 years after surgery are 90% and 80%, and 70% and 50%, respectively.[10] In advanced disease, however, this therapeutic alternative is contraindicated, as there is no evidence that extended surgery can improve the prognosis,[11] which leaves the option of using somatostatin analogs for disease stabilization.[10] In the case of hepatic and lymph node metastases, surgical removal of tumors is primarily intended to relieve symptoms.[1,10] The use of adjuvant chemotherapy and radiotherapy is controversial in the literature[10,11] and there is no consensus. Nevertheless, there are reports of patients with atypical carcinoid tumors who would benefit from these treatments.[11]

Resumo

Tumor carcinoide endobrônquico: aspectos radiológicos em um caso clínico

Descrevemos um caso de paciente do gênero feminino, 21 anos, apresentando crises de dispneia e sibilância há 2 anos. Radiografia torácica evidenciou perda volumétrica do lobo inferior esquerdo e opacidade triangular basal retrocardíaca ipsilateral. Tomografia computadorizada mostrou

formação expansiva sólida com ápice protruindo para o interior dos brônquios principal e lobar inferior esquerdos, promovendo atelectasia distal. Estudos anatomopatológico e imuno-histoquímico após biópsia transbrônquica da lesão diagnosticaram um tumor carcinoide típico, confirmado após ressecção tumoral com pneumectomia total esquerda.

Palavras-chave: Tumor Carcinoide. Endobrônquico. Pulmonar. Típico. Diagnóstico por Imagem.

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Peliosis hepatis and systemic lupus erythematosus: A rare condition identified by magnetic resonance imaging

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Summary

Peliosis hepatis is a rare benign disorder characterized by the presence of multiple cavities filled with blood with no preferential localization in the liver parenchyma. It may be related to several etiologic conditions, especially infections and toxicity of immunosuppressive drugs. To our knowledge, there are only three articles reporting the association between peliosis hepatis and systemic lupus erythematosus. In this report, we describe a case of this rare condition, highlighting the importance of magnetic resonance imaging. A short review of this subject is also presented.

Keywords: Peliosis Hepatis. Magnetic Resonance Imaging. Liver Diseases. Lupus Erythematosus, Systemic.

Introduction

Peliosis hepatis (PH) is characterized by multiple cavities filled with blood scattered throughout liver parenchyma.[1] There are many conditions associated with it, including infectious diseases (tuberculosis, acquired immunodeficiency syndrome), immunological disorders (post-transplant immunodeficiency), cancer (hematologic malignancies, hepatocellular carcinoma) and drugs (anabolic steroids, oral contraceptives and immunosuppressive drugs such as glucocorticoids, azathioprine and cyclosporine).[2] However, etiology is unknown in up to 50% of cases.[3]

To our knowledge, only three case reports linking PH to systemic lupus erythematosus (SLE) were published so far.[4-6] Although changes in liver tests are common in patients with SLE, in most cases the liver has a normal morphology.[4,5] In this case, we highlight the role of magnetic resonance imaging in the identification of PH in a patient with SLE.

Case report

In 2010, a 59-year-old white female SLE patient presented at our institution with upper abdominal pain for the past 6 months. The pain was mild, intermittent and not related with feeding. She was diagnosed with lupus when she was 19 years old, having symptoms of arthritis, nephritis, transverse myelitis, thrombocytopenia and cutaneous manifestations. Antinuclear antibodies tested positive (1/1,280, homogeneous nuclear pattern) as well as anti-dsDNA antibodies. She was exposed to high doses of prednisone, intravenous cyclophosphamide, azathioprine and dapsone. She is currently being treated with prednisone 7.5 mg daily and mycophenolate mofetil 1 g bid. She denied alcohol abuse, smoking or use of illicit drugs.

At the time of this evaluation, laboratory tests showed: hemoglobin 16.8 mg/dL, leukocytes 14,770/mm^3 without immature forms, platelets 73.000/mm^3 (reference: 150,000-450,000/mm^3), creatinine 1.39 mg/dL (creatinine clearance 45 mL/min), erythrocyte sedimentation rate 2 mm/hour, C-reactive protein < 3 mg/dL, C3 and C4 within normal range. Liver function panel revealed aspartate aminotransferase 48 U/L (reference: < 31 U/L), alanine aminotransferase 60 U/L (reference: < 31 U/L), total bilirubin, gamma-glutamyl transferase, alkaline phosphatase, albumin and coagulation within normal limits. She was negative for viral hepatitis and for anti-smooth muscle antibody and anti-LKM.

Abdominal ultrasonography revealed multiple nodules smaller than 1 cm, slightly hyperechoic, with imprecise margins and a diffuse distribution throughout the liver parenchyma, most evident in the left lobe. Magnetic resonance imaging (MRI) of the upper abdomen showed numerous tiny nodes with high signal intensity on T2 and progressive enhancement by contrast, most evident in the late phase.

After 3 years of follow-up, a new MRI showed an increase in the number of nodular formations previously identified (Figure 1). During this period, she spontaneously recovered from abdominal pain and the liver function panel remained unchanged.

Discussion

PH was first described by Schoenlank in 1916.[7] Since then, the pathogenesis of PH remains unclear, although several hypotheses have been proposed. It seems to be related to obstruction of sinusoidal blood flow, direct injury of sinusoidal barrier and focal hepatocellular necrosis.[8] Peliotic cysts may present with variable morphology and size, ranging from smaller than a millimeter to several centimeters. The mean length of the cavities seems predictive of hepatomegaly and portal hypertension.[9]

It may be asymptomatic and found incidentally only at necropsy.[10] Matsumoto et al.[4] did a histopathological evaluation of 52 livers from patients with SLE (51 necropsies and one surgery). In this study, PH was found in six patients, three of whom had received high doses of glucocorticoids, which could be related to the onset of the lesions.

Langlet et al.[5] described a severe acute pancreatitis associated with PH in an SLE patient. In this report, pancreatitis and liver involvement were ascribed to widespread vasculitis and the patient was treated with pulse methylprednisolone, followed by azathioprine. Despite the chronic use of these drugs possibly being involved with the development of PH, the authors described the reduction of peliotic lesions after treatment.

MRI of uncomplicated PH may reveal mild lesions with high signal intensity on T2-weighted images due to subacute bleeding.[3,9] Other possible findings include hepatomegaly, signs of acute liver failure and breakage of larger peliotic lesions resulting in severe intraperitoneal hemorrhage and shock. [11,12]

Despite the appearance of the MRI lesions described in this report being similar to other vascular lesions (e.g. hemangiomas), diffuse distribution and temporal progression strongly suggest PH. An important differential diagnosis is hepatic hemangiomatosis, but that would present with confluent infiltrative lesions, often associated with hepatomegaly. Biliary hamartomas are similar to PH on T2-weighted images, however, they usually do not exhibit enhancement and do not progress significantly throughout the years.[13-15]

PH is a rare vascular condition that should encompass the differential diagnosis of atypical liver damage in patients with SLE. Liver biopsy remains the gold standard diagnostic test; nonetheless, given the risk of bleeding, non-invasive diagnostic tools such as MRI are often used to differentiate PH from other unusual hepatic conditions.

Resumo

Peliose hepática e lúpus eritematoso sistêmico: uma rara condição identificada pela ressonância nuclear magnética

Peliose hepática é uma patologia benigna rara caracterizada pela presença de múltiplas cavidades preenchidas por sangue sem localização preferencial no parênquima do fígado. Pode estar relacionada a uma série de condições etiológicas, dentre elas doenças infecciosas e toxicidade por drogas imunossupressoras. Para nosso conhecimento,

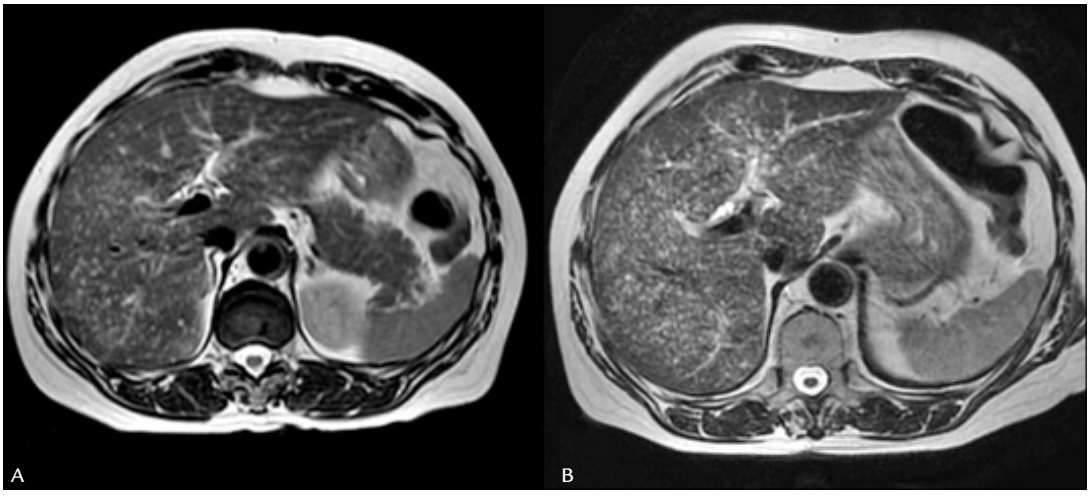

FIGURE 1 Axial T2-weighted MRI: multiple nodular hyperintense lesions that progressed along the three years of follow-up: 2010 (panel A) to 2013 (panel B).

existem apenas três artigos que abordam a associação entre peliose hepática e lúpus eritematoso sistêmico. Neste relato, descrevemos um caso desta rara condição, destacando a importância da ressonância magnética. Uma breve revisão sobre o tema é apresentada.

Palavras-chave: Peliose Hepática. Imagem por Ressonância Magnética. Hepatopatias. Lúpus Eritematoso Sistêmico.

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Systemic administration of curcumin nanoparticles protects ischemia-reperfusion injury in ovaries: An animal model study

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Summary

Objective: Ovarian torsion must be diagnosed and treated as early as possible. The aim of the present study was to investigate the effects of intraperitoneal administration of nanocurcumin on ischemia-reperfusion injury in ovaries.
Method: Thirty-five (35) healthy female Wistar rats weighing approximately 250 g were randomized into seven experimental groups (n=5): Group SSG – The rats underwent only laparotomy. Group I: A 3-hour ischemia only. Group I/R: A 3-hour ischemia and 3-hour reperfusion. Group I/C: A 3-hour ischemia only, and 1 mg/kg intraperitoneal administration of curcumin 2.5 hours after induction of ischemia. Group I/R/C: A 3-hour ischemia, 3-hour reperfusion, and 1 mg/kg intraperitoneal administration of curcumin 2.5 hours after induction of ischemia. Group I/NC: A 3-hour ischemia only and 1 mg/kg intraperitoneal administration of nanocurcumin 2.5 hours after induction of ischemia. Group I/R/C: A 3-hour ischemia, 3-hour reperfusion and 1 mg/kg intraperitoneal administration of nanocurcumin 2.5 hours after induction of ischemia.
Results: Nanocurcumin-treated animals showed significantly improved development of ischemia and reperfusion tissue injury compared to those in the other groups ($p<0.05$). Significant higher values of SOD, tGSH, GPO, GSHRd and GST were observed in I/R/NC animals compared to those in the other groups ($p<0.05$). The damage indicators (NOS, MDA, MPO and DNA damage level) were significantly lower in I/R/NC animal compared to those of other groups ($p<0.05$).
Conclusion: Intraperitoneal administration of nanocurcumin can be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia.

Keywords: Curcumin. Nanoparticles. Ovary.

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Introduction

There are various conditions, such as long mesovarium and adnexal venous congestion, that could result in torsion of ovary and subsequently obstruction of the ovarian vessels. This causes a life-threatening reduction in tissue blood flow and permanent tissue damage.[1] Therefore, ovarian torsion must be diagnosed and treated as early as possible to preserve ovarian function and prevent future infertility.[2] Upon detection of ovarian torsion, detorsion of the twisted adnexa and evaluation of tissue reperfusion is proposed to prevent future infertility even in case of cyanotic tissues.[2,3] This ovarian torsion-detorsion process is named ischemia-reperfusion injury.[4]

Reperfusion of the ischemic tissue leads to much more serious damage to the tissue than the damage caused by ischemia.[5] Reperfusion-related damage in the cell is created by many factors, mostly including oxygen-derived free radicals, which are rapidly generated in the tissue as a result of reperfusion.[6] Due to physiological or pathological alterations, oxidative damage takes place with changes favoring the oxidation process.[7] Prompt diagnosis to reduce ischemic and reperfusion injury and its consequences is

still unachievable with this approach. Therefore, studies on prevention of reperfusion injury seem very important.[8]

A proposed pathogenesis for tissue injury during reperfusion is the accumulation of activated neutrophils that release reactive oxygen species.[9] Lipid peroxidation in the cell is the most deleterious effects of free radicals that ultimately reduce the membrane potential and subsequently cause cell injury. Malondialdehyde (MDA), one of the end products of lipid peroxidation, also results in severe cell damage by inducing polymerization and cross linking in membrane components.[10] Free oxygen radicals react with DNA and form 8-hydroxyguanine (8-OHGua), which is one of the products of DNA damage.[11] In spite of the fact that generation of free oxygen radicals occurs continuously in cells, the presence of endogenous antioxidant defense systems preserves tissues from the harmful effects of free oxygen radicals.[12] Various agents, anti-inflammatory and antioxidant free radical scavengers have been reported with promising beneficial effects on prevention of ischemic/reperfusion injuries in tissues.[13-16]

Curcumin is the main phenolic pigments extracted from turmeric, the powdered rhizome of *Curcuma longa*, along with demethoxycurcumin and bisdemethoxycurcumin.[17] Extensive research indicates that curcumin possesses potent antioxidant and anti-inflammatory properties, and inhibits lipid peroxidation and scavenges superoxide anion, singlet oxygen, nitric oxide and hydroxyl radicals.[18-20] Administration of curcumin has been reported to be effective in reversing tissue damage induced by ischemia reperfusion injury in ovarian torsion.[21]

Curcumin, a naturally-occurring polyphenolic compound, is considerably promising; however, its poor water solubility and fast degradation profile compromise its bioavailability way below the threshold level on administration. Over a period of time, strong emphasis has been given to improve the biodistribution of native curcumin, but only recently the application of the field of nanotherapeutics has significantly improved its therapeutic efficacy. This is through the development of nanorange formulations of curcumin, popularly known as the nanocurcumin.[22]

The physiologic characteristic of the peritoneal cavity, which helps remove toxic metabolites from the body, has been successfully exploited to provide peritoneal dialysis in end stage renal disease patients.[23] The same characteristics of the peritoneal membrane also provide a useful doorway in the body for several pharmacological agents. One advantage would be that the drug achieves therapeutic efficacy in the target site while minimizing the systemic toxicities. Intraperitoneal administration seems more effective and may increase drug availability if oral administration poses any difficulties. It is clear that transperitoneal absorption of the drug is much faster than oral administration.[24]

The present study was different from other studies in the literature for using nanocurcumin on ischemia/reperfusion injury. Aimed to study peritoneal effects of nanocurcumin on ischemia/reperfusion injury, our study was designed to determine if nanocurcumin could in fact protect against ischemia/reperfusion-induced ovarian damage. The assessments were based on histopathological and biochemical parameters.

Method

Study design and animals

Two weeks before and during the experiments, the animals were housed in individual plastic cages at room temperature (23±3°C), stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups. The present study was designed and modified based on a method described by Oral et al., 2010. Thirty-five (35) healthy female Wistar rats weighing approximately 250 g were randomized into seven experimental groups (n=5): SSG (SSG Surgery Group) – The rats underwent only laparotomy; group I – A 3-hour ischemia only; group I/R – A 3-hour ischemia and a 3-hour reperfusion; group I/C – A 3-hour ischemia only and 100 mg/kg intraperitoneal administration of curcumin (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) 2.5 hours after induction of ischemia; group I/R/C – A 3-hour ischemia, a 3-hour reperfusion and 100 mg/kg intraperitoneal administration of curcumin 2.5 hours after induction of ischemia; group I/NC – A 3-hour ischemia only and 1 mg/kg intraperitoneal administration of nanocurcumin (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) 2.5 hours after induction of ischemia; group I/R/NC – A 3-hour ischemia, a 3-hour reperfusion and 1 mg/kg intraperitoneal administration of nanocurcumin 2.5 hours after induction of ischemia. The right ovaries were transferred to a 10% formaldehyde solution for histopathological assessments and the left ovaries were dissected free of surrounding soft tissues and then stored in a freezer at -80°C for biochemical assessments.

Preparation of nanocurcumin

Nanocurcumin was prepared using a method previously described by other authors. In brief, curcumin (100 mg, 0.27 mmol) was placed in dichloromethane (20 mL), and

1 mL of this solution was sprayed into boiling water (50 mL) dropwise with a flow rate of 0.2 mL/min for 5 minutes under ultrasonic conditions, with an ultrasonic power of 100 W and a frequency of 30 kHz. After sonication for 10 minutes, the contents were stirred at 200-800 rpm at room temperature for about 20 minutes when a clear orange-colored solution was obtained. The solution was concentrated under reduced pressure at 50°C and then freeze-dried to obtain an orange powder. A co-TLC of the powdered sample with standard curcumin showed both to have the same Rf values. 1H NMR and ultraviolet (UV) spectra of the lyophilized powder confirmed it to be curcumin. Maintaining the drop flow was significant for both forming nanoparticles and maintaining size uniformity. The mean particle diameter of curcumin nanoparticles was measured by dynamic light scattering (DLS) performed on Malvern Zetasizer S90 series. The sample was prepared by taking 1 mg of the lyophilized nanocurcumin powder in 10 mL of distilled water. Transmission electron micrograph (TEM) analysis was performed on a Morgagni 268 D from FEI. The sample was prepared by placing a drop of the aqueous dispersion of curcumin nanoparticles on the copper grid and allowing it to air dry. Scanning electron micrograph (SEM) of the aqueous dispersion was recorded on a Jeol JSM 840 microscope by spreading the nanoparticles dispersion over a carbon tape and drying it under nitrogen stream. The sample was then coated in a sputter coater (EMITECH K 550 x) with a gold layer under vacuum conditions.[25]

Surgical procedure

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[26] The Ethical Committee of the Urmia University of Medical Sciences approved all the experiments.

A longitudinal midline incision was made in the lower abdomen and the uterine horns and adnexa were exposed. In order to induce ischemia, a vascular clamp was applied on the rats' ovary vessels. After a 3-hour period of ischemia, both ovaries were surgically dissected for histopathological and biochemical assessments. To induce ischemia/reperfusion, both ovaries underwent ischemia the same way and, at the end of a 3-hour period, the vascular clamps were chosen, removed and a 3-hour reperfusion was obtained. Then, the ovaries were dissected for histopathological and biochemical assessments.

Histopathological assessments

The ovaries were fixed in 10% buffered formalin for 24 hours. The tissue samples were then processed and embedded in paraffin (5-µm semithin sections). The samples were then dewaxed, rehydrated and stained routinely with hematoxylin and eosin. The sections were then observed under a light photomicroscope. For semithin sections, ovaries were fixed in 2.5% buffered glutaraldehyde and postfixed in 2% OsO_4 for 2 h, dehydrated in a graded ethanol series and embedded in epon resin. Semithin transverse sections (5 µm) were next stained with toluidine blue and examined under a light microscope.

Biochemical assessments

Tissue processing for biochemical assessments of ovaries

The ovary tissue samples were kept at -80°C for 3 days, and then enzyme activities were determined in rat ovary tissues. The ovary tissues were ground under liquid nitrogen using a mortar. One half gram was weighed for each group and then treated with 4.5 mL of an appropriate buffer. This mixture was homogenized on ice with use of an ultra-turrax homogenizer (IKA, Werke, Germany) for 15 minutes. Homogenates were filtered and centrifuged by using a refrigerator centrifuge at 4°C. Then the supernatants were used to determine the enzymatic activities. All assays were carried out at room temperature.

Superoxide dismutase (SOD) analysis

Superoxide dismutase estimation was based on the generation of superoxide radicals produced by xanthine and the xanthine oxidase system, which reacts with nitroblue tetrazolium to form formazan dye.[27] Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction and is expressed as millimoles per minute per milligram of tissue.

Nitric oxide synthase (tNOS) activity

Nitric oxide synthase activity of rat ovaries was measured spectrophotometrically using the oxidation of oxyhemoglobin to methemoglobin by NO as described by other authors.[28] The absorption difference between 401 and 421 nm was continuously monitored with a dual wave length recording spectrophotometer at 37°C. For the total NOS (tNOS) assay, the incubation medium contained 1.6 mmol/L oxyhemoglobin, 200 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 100 mmol/L L-arginine, 100 mmol/L of the reduced form of nicotinamide-adenine dinucleotide phosphate, 40 mmol/L potassium phosphate (pH 7.2), 1 mmol/L NG-nitro-L-arginine and 10% (vol/vol) tissue extract with 50 mmol/L L-valine to inhibit arginase.[29]

Malondialdehyde (MDA) analysis

Concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test.[30] The rat ovaries were rinsed with cold saline. The corpus mucosa was scraped, weighed and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 2-thiobarbiturate (1.5 mL of 8 g/L), acetic acid (1.5 mL of 200 g/L), sodium lauryl sulfate (0.2 mL of 80 g/L) and distilled water (0.3 mL). The mixture was incubated at 98°C for 1 hour. n-butanol: pyridine 5 mL (ratio:15:l) was then added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4,000 rpm. Absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane.

Myeloperoxidase (MPO) analysis

The activity of MPO in the total homogenate was measured according to previously described methods.[31] The sample was weighed and homogenized in 2 mL of 50 mmol/L phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide (HDTMAB) and centrifuged at 3,500 rpm for 60 min at 4°C. The supernatant was used to determine MPO activity using 1.3 mL 4-aminoantipyrine-2% phenol (25 mM) solution. 25 mmol/L 4-aminoantipyrine–2% phenol solution and 0.0005% 1.5 mL H_2O_2 were added and equilibrated for 3-4 minutes. After establishing the basal rate, a sample suspension (0.2 mL) was added and mixed. Increases in absorbance at 510 nm for 4 min at 0.1-min intervals were recorded. Absorbance was measured at 412 nm.

Total glutathione (tGSH) analysis

The amount of GSH in the total homogenate was measured according to the previously described methods, with some modifications.[32] The sample was homogenized at pH 7.5, in Tris-HCl buffer (2 mL of 50 mmol/L). The homogenate was precipitated with trichloroacetic acid (0.1 mL of 25%), and the precipitate was removed after centrifugation at 4,200 rpm at 4°C for 40 minutes; the supernatant was used to measure GSH level. A total of 1,500 µL of measurement buffer (200 mmol/L Tris-HCl buffer containing 0.2 mmol/L EDTA at pH 7.5), 500 µL supernatant, 100 µL DTNB (10 mmol/L) and 7,900 µL methanol were added to a tube and vortexed and incubated for 30 minutes at 37°C. 5,5-dithiobis (2- nitrobenzoic acid) (DTNB) was used as a chromogen; it formed a yellow-colored complex with sulfhydryl groups. Absorbance was measured at 412 nm using a spectrophotometer (Beckman DU 500, USA). The standard curve was obtained using reduced glutathione.

Glutathione peroxidase (GPO) analysis

GPO activity was determined according to the method of Lawrence and Burk.[33] After tissue homogenization, supernatant was used for GPO measurement. Following the addition of KH_2PO_4, EDTA, GSH, B-NADPH, NaN_3 and GR, the mixture was incubated. As soon H_2O_2 was added, the chronometer was turned on and the absorbance at 340 nm was recorded for 5 minutes every 15 seconds.

Glutathione reductase (GSHRd) analysis

GR activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm according to Carlberg and Mannervik method.[34] After tissue homogenization, supernatant was used for GR measurement. After NADPH and GSSG were added, a chronometer was set on and absorbance was measured for 5 minutes with 30 minutes intervals at 340 nm spectrophotometrically.

Glutathione S-transferase (GST) activity

GST activity was determined by Habig and Jakoby.[35] Enzyme activity was determined in a 4-mL cuvette containing 30 mM GSH, 30 mM 1-chloro-2,6-dinitrobenzene, 0.1 M PBS (pH 6.5), and tissue homogenate at 340 nm using a spectrophotometer.

Isolation of DNA from ovarian tissue

DNA isolation was performed using a method previously described by other authors.[8] In brief, the tissue samples were homogenized at 4°C in 1 mL of homogenization buffer (0.1 M NaCl, 30 mM Tris, pH 8.0, 10 mM EDTA, 10 mM 2-mercaptoethanol, 0.5% (v/v) Triton X- 100) with six passes of a Teflon-glass homogenizer at 200 rpm. The samples were centrifuged at 4°C for 10 min at 1,000 g to pellet nuclei. The supernatant was discarded, and the crude nuclear pellet re-suspended and re-homogenized in 1 mL of extraction buffer (0.1 M Tris, pH 8.0, 0.1 M NaCl, 20 mM EDTA) and re-centrifuged as above for 2 min. The washed pellet was re-suspended in 300 µL of extraction buffer with a wide orifice 200 µL Pipetman tip. The re-suspended pellet was subsequently incubated at 65°C for 1 hour with the presence of 0.1 mL of 10% SDS, 40 µL proteinase K, and 1.9-mL leukocyte lysis buffer. Then, ammonium acetate was added to the crude DNA sample to yield a final concentration of 2.5 mol/L, and centrifuged in a micro centrifuge for 5 minutes. The supernatant was removed and mixed with two volumes of ethanol to precipitate the DNA fraction. After centrifugation, the pellet was dried under reduced pressure and dissolved in sterile water. The absorbance of this fraction was measured at 260 and 280 nm. Purification of DNA was determined as A 260/280 ratio 1.8.

cDNA hydrolysis with formic acid

DNA hydrolysis with formic acid was performed based on a modified method described by other authors.[8] Briefly, 50 mg of DNA were hydrolyzed with 0.5 mL of formic acid (60%, v/v) for 45 minutes at 150°C. The tubes were allowed to cool. The contents were then transferred to Pierce micro-vials, covered with Kleenex tissues cut to size, secured in place using a rubber band and cooled in liquid nitrogen. Formic acid was removed by freeze-drying and prior to analysis by HPLC they were re-dissolved in the eluent, final volume 200 µL.

Measurement of 8-hydroxy-2 deoxyguanine (8-OH Gua)

Measurement of 8-hydroxy-2 deoxyguanine (8-OH Gua) was performed based on a modified method described by others.[8] Briefly, the amount of 8-OH gua and guanine (Gua) was measured using a HPLC system equipped with an electrochemical detector, HP Agilent 1,100 module series and E.C.D. HP 1049 A. The amount of 8-OH gua and Gua was analyzed on a 250 4.6 mm Supelco LC-18-S reverse-phase column. The mobile phase was 50 mM potassium phosphate, pH 5.5, with acetonitrile, a 97 volume acetonitrile and a 3 volume potassium phosphate, and the flow rate was 1.0 mL/min. The detector potential was set at 0.80 V for measuring the oxidized base. Gua and 8-OH Gua (25 pmol) were used as standards. The 8-OH gua levels were expressed as the number of 8-OH gua molecules/105 Gua molecules.

Statistical analysis

Experimental results were expressed as mean±SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using repeated measures and a factorial ANOVA with two between-subject factors. Bonferroni test for pairwise comparisons was used to examine the effect of time and treatments. The differences were considered significant when $p<0.05$.

Results

Histopathological findings

The histologic design of the ovarian tissue in the SSG animals was normal. Ovarian tissues in the ischemia group showed condensed hemorrhage and severe vascular congestion along with degenerative and necrotic changes in many of the cells. The tissues in the I/R group showed histopathological changes of condensed hemorrhage, infiltration of inflammatory cells along with degenerative and apoptotic cells. Polymorphonuclear leukocytes (neutrophils) were dominant cell types. In I/R/C group general histologic and cellular structures of the tissues were not normal in appearance; however, mild vascular congestion and edema were observed. In I/R/NC group only a slightly mild hemorrhage was around ovarian follicles. The general histologic structure of the ovarian tissue in this group was normal and no important pathologic findings in the structural level were observed except for only a slightly mild inflammation, vascular congestion and edema (Figure 1).

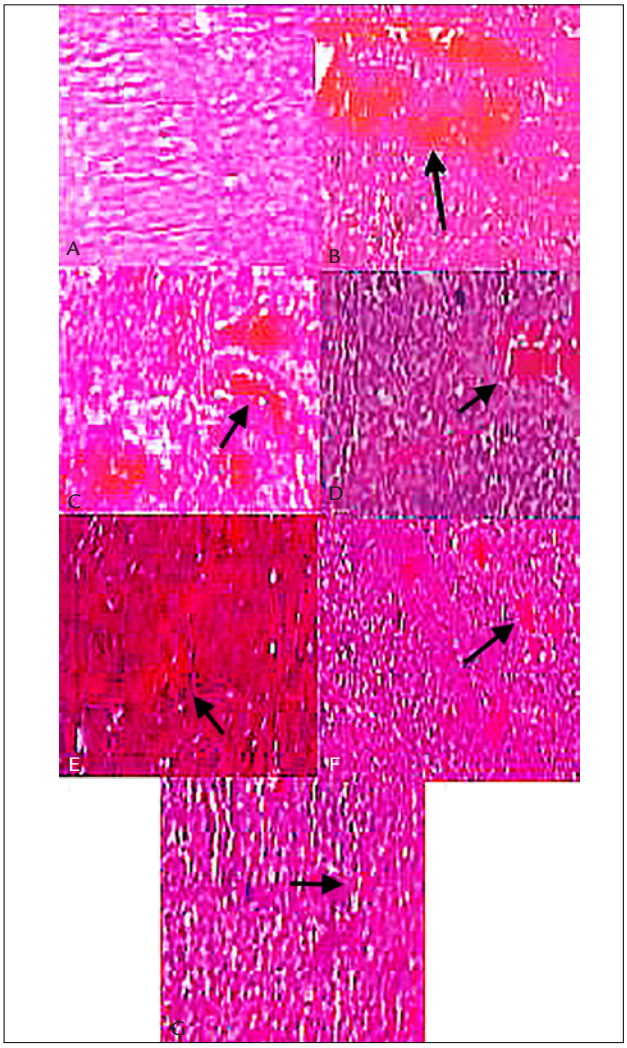

FIGURE 1 Histologic micrographs of ovarian tissue in SSG (A), I (B), I/R (C), I/C (D), I/R/C (E), I/NC (F) and I/R/NC (G) groups. Micrograph B shows condensed hemorrhage and severe vascular congestion (arrow), as well as severe edema (arrowhead). Micrograph C shows condensed hemorrhage and vascular congestion (arrow), as well as edema (arrowhead). Micrograph D shows moderate vascular congestion (arrow) and moderate edema (arrowhead). Micrograph E shows mild vascular congestion and edema (arrow). Micrographs F and G show slightly mild vascular congestion and edema (arrows). Scale bar: 200 µm.

The numerical densities of neutrophils were also estimated at $15 \times 10^{-6}/\mu m^3$, $11 \times 10^{-6}/\mu m^3$, $17 \times 10^{-6}/\mu m^3$, $16 \times 10^{-6}/\mu m^3$ and $12 \times 10^{-6}/\mu m^3$, $15 \times 10^{-6}/\mu m^3$, $10 \times 10^{-6}/\mu m^3$ in SSG, I, I/R, I/C, I/R/C, I/NC and I/R/NC groups, respectively.

Biochmical findings

Superoxide dismutase (SOD) analysis

The value of SOD activity was 69.5±0.57 mmol/min/mg tissue in the SSG group. The values of SOD declined to 35.8±0.22 and 57.2±0.21 mmol/min/mg tissue in I and I/R groups, respectively. However, intraperitoneal administration of 1 mg/kg of nanocurcumin reversed the trend and increased the activity of SOD to 78.2±0.31 mmol/min/mg tissue in the ovarian tissue in I/R/NC group. The value of SOD activity in I/R/NC group was significantly higher than those of the other experimental groups ($p<0.05$) (Table 1).

Nitric oxide synthase (NOS) activity

The value of tNOS activities was increased in I and I/R groups and was significantly higher than those of SSG group ($p<0.05$). However, intraperitoneal administration of 1 mg/kg of nanocurcumin reversed the trend and decreased tNOS activity in the rats' ovary. In I/R/NC group the value of tNOS activity was significantly lower than those of the other experimental groups ($p<0.05$) (Table 1).

Malondialdehyde (MDA) analysis

The results of the present study showed that concentration of MDA in SSG group was 5.7±0.19 µmol/g protein in ovarian tissue. MDA level in I/R group was significantly increased to 11.6±0.23 µmol/g protein ($p<0.01$). Intraperitoneal administration of nanocurcumin significantly decreased the levels of MDA in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

Myeloperoxidase (MPO) analysis

The level of MPO was significantly increased in I and I/R groups ($p<0.05$). Intraperitoneal administration of nanocurcumin reversed the trend and significantly decreased the levels of MPO in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

Total glutathione (tGSH) analysis

The values for tGSH levels were 9.8±0.33 and 4.9±0.31 nmol/g protein in SSG and I/R animals, respectively. Intraperitoneal administration of nanocurcumin significantly increased the levels of GSH in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

Glutathione peroxidase (GPO) analysis

The values for GPO levels were 38.6±2.64 and 17.6±1.41 u/g protein in SSG and I/R animals, respectively. Intraperitoneal administration of nanocurcumin significantly increased the levels of GPO in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

Glutathione reductase (GSHRd) analysis

The GSHRd activities in ovarian tissue in the SSG and I/R animals were 33.5±3.26 and 16.4±1.27 u/g protein, respectively. Intraperitoneal administration of nanocurcumin significantly increased the levels of GSHRd in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

TABLE 1 Comparison of the activities of SOD, NOS, MDA, MPO, GSH, GPO, GSHRd, GST and a DNA damage product of 8-OHGua/Gua in the ovarian tissues of the animals of the all experimental groups. Data are expressed as mean±SD.

Variables	Group SSG	Group I	Group I/R	Group I/C	Group I/R/C	Group I/NC	Group I/R/NC
SOD (mmol/min/mg)	69.5±0.57	35.8±0.22	57.2±0.21	53.6±0.38	73.3±0.22	61.3±0.42	78.2±0.31*
NOS (nmol/min/mg)	3.8±0.12	3.9±0.15	3.9±0.55	3.2±0.12	3.1±0.24	3.3±0.13	3.4±0.14*
MDA (µmol/g protein)	5.7±0.19	12.8±0.24	11.6±0.23	9.6±0.35	6.8±16	8.3±0.63	5.4±15*
MPO (U/g protein)	6.5±0.18	16.7±0.43	13.9±0.28	12.8±0.21	8.7±0.48	10.7±0.12	7.1±0.31*
tGSH (nmol/g protein)	9.8±0.33	2.9±0.22	4.9±0.31	5.2±0.12	7.3±0.19	6.4±0.22	8.1±0.13*
GPO (U/g protein)	38.6±2.64	12.7±2.48	17.6±1.41	20.7±1.52	29.5±2.54	22.4±1.31	3.25±2.66*
GSHRd (U/g protein)	33.5±2.26	9.5±1.83	16.4±1.27	19.9±1.82	24.0±2.30	21.2±1.12	28.5±2.53*
GST (U/g protein)	21.6±1.21	10.2±1.27	14.7±1.38	16.1±1.21	19.7±1.11	17.3±1.08	20.5±1.21*
8-OHGua/Gua (pmol/L)	1.3±0.14	2.7±0.15	2.3±0.15	1.7±0.13	1.5±0.11	1.5±0.12	1.3±0.14*

I: ischemia; I/R: ischemia-reperfusion; I/Nimodipine: ischemia plus intraperitoneal administration of nimodipine; I/R/Nimodipine: ischemia plus reperfusion plus intraperitoneal administration of nimodipine; SOD: superoxide dismutase; NOS: nitric oxide synthase; MDA: malondialdehyde; MPO: myeloperoxidase; tGSH: total glutathione; GPO: glutathione peroxidase; GSHRd: glutathione reductase; GST: glutathione S-transferase; 8-OHGua/Gua: 8-hydroxy-2 deoxyguanine.
*p<0.0 vs. other experimental groups.

Glutathione S-transferase (GST) activity

The GST activities in ovarian tissue in the SSG and I/R animals were 21.6±1.21 and 14.7±1.38 u/g protein, respectively. Intraperitoneal administration of nanocurcumin significantly increased the levels of GST in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

Measurement of 8-hydroxy-2 deoxyguanine (8-OH Gua)

The levels of 8-OHGua/Gua, a DNA damage product, were 1.3±0.14 and 2.3±0.15 pmol/L in SSG and I/R animals, respectively. Intraperitoneal administration of nanocurcumin significantly decreased the levels of GSHRd in ovarian tissues of I/R/NC animals ($p<0.05$) (Table 1).

Discussion

The present study investigated whether intraperitoneal administration of nanocurcumin is useful in the prevention of ovarian damage in ischemia/reperfusion conditions in rat ovaries and its results revealed beneficial effects. Histopathological and biochemical assessments were performed in SSG, ischemia, ischemia-reperfusion, ischemia-controlled plus IP administration of nanocurcumin groups.

Histopathological, edema, vascular congestion, hemorrhages and leukocyte infiltration parameters were used. Biochemically, the activities of SOD, NOS, MDA, MPO, GSH, GPO, GSHRd, GST and a DNA damage product of 8-OHGua/Gua were assessed in the ovarian tissues of the animals in all of the experimental groups.

Ischemia, ischemia-reperfusion and intraperitoneal nanocurcumin applied to tissues were analyzed histopathologically. Results showed that oxidative stress level followed a parallelism with tissue damage. Edema, vascular congestion, hemorrhages and leukocyte infiltration have been used as histopathological parameters in the evaluation of cell condition.[36] Edema, vascular congestion, hemorrhage and leukocyte infiltration in the I/R/NC animals were milder than in the I/R/C group.

In the present study, the levels of SOD in ovarian tissue were assessed and compared in all the experimental groups. SOD activity in SSG and IR/NC showed no significant difference. SOD is an antioxidant enzyme that catalyzes the conversion of superoxide free radical into hydrogen peroxide and molecular oxygen. SOD and endogenous antioxidant enzymes neutralize free radicals and protect tissues from the harmful effects of free radicals and active oxygen species.[37] Our results showed that, in the I/R/NC animals, SOD was increased compared to those in I/C and I/R/C groups and intraperitoneal administration of nanocurcumin protected the ovarian tissue against ischemia-reperfusion injury.

It has been demonstrated that hypoxia generates iNOSs, which plays an important damaging role in I/R injury.[38] iNOS is increased after cellular stimulation via cytokines in macrophages, neutrophils, and microglia and may also contribute to late-stage tissue injury.[39] iNOS derives primarily from the polymorphonuclear neutrophilic leukocytes during reperfusion, and down-regulation of iNOS could limit cell injury caused by hypoxia.[40,41] Our findings showed that the iNOS levels in ovarian tissue of I and I/R rats were increased compared to those of the SSG animals. Down-regulation of iNOS could limit cell injury caused by hypoxia. Our results showed that, in the I/R/NC animals, iNOS was down-regulated compared to those in I/C and I/R/C. Thus, intraperitoneal administration of 1 mg/kg nanocurcumin protected ovarian tissue against ischemia-reperfusion injury to a greater extent than 100 mg curcumin.

MDA is a lipid peroxidation product and occurs as a result of the peroxidation of fatty acids that contain three or more double bonds. MDA causes cross-linking of membrane components and leads to negative consequences such as changes in ion permeability and enzyme activity by affecting ion exchange through the cell membranes.[42,43] MDA levels in the present study were found to be much lower in I/R/NC animals compared to those in the other experimental groups. This could protect the tissues against ischemia-reperfusion injury in nanocurcumin-treated animals.

MPO is produced by neutrophils and macrophages, it catalyzes the reaction between hydrogen peroxide and chlorine and results in the toxic compound hypochlorous acid. Hypochlorous acid is involved in the formation of the hydroxyl radical.[44,45] It has been demonstrated that MPO activity is increased in ischemia-reperfusion induced ovarian tissue.[46] This finding was in agreement with results of our study. MPO activity was suppressed in nanocurcumin-treated animals of our study.

GSH is an antioxidant used to measure oxidative stress. Reperfusion after ischemia is reported to cause severe damage to ovarian tissue and suppress the GSH levels.[36] GSH plays a role in the protection of cells against oxidative stress and toxic compounds as well as the metabolic processing of many endogenous compounds such as estrogen, prostaglandin and leukotrienes.[47] GSH, as an antioxidant, reacts with peroxides and free radicals and converts them into harmless products and subsequently protects the cells against the potential oxidative damage of free radicals. These findings were in agreement with our results. We found that oxidative stress was minimized and the severe damage due to sudden reperfusion was

prevented in nanocurcumin-treated animals to a greater extent than seen in curcumin-treated animals.

GPO activity is significantly reduced in tissues undergoing oxidative stress-related conditions, as in ischemia-reperfusion injury.[48] GPO detoxifies the hydrogen peroxide radical that forms in the cell by converting it to water and prevents the formation of more toxic products from hydrogen peroxide radical.[49] In the present study, a significant decrease in GPO activity was observed in ovarian tissues of I/R/NC animals.

GSH is oxidized during the detoxification of hydrogen peroxide radical. GSHRd is a NADPH-dependent enzyme that converts oxidized glutathione to reduced glutathione.[50] GSHRd is reported to show higher activity in healthy tissue and, in parallel with tissue damage, its activity is decreased.[51] In our study, activity of GSHRd was significantly increased in nanocurcumin-treated animals compared to those in I/C and I/R/C groups.

GST binds foreign substances to the –SH group of cysteine in glutathione, neutralizes the electrophilic regions and protects the cells from the harmful effects of foreign substance regions.[52] Activity of GST has been reported to be suppressed in oxidative tissue injury induced by ischemia.[52] Consistently, our findings showed that GST activity in ovarian tissue of nanocurcumin-treated animals was significantly lower than those in I/C and I/R/C groups.

DNA molecules are damaged if free radicals are in close proximity to the DNA molecules.[53] Hydroxyl radical reacts very easily with deoxyribose and the bases, causing DNA damage through extracting hydrogen from nucleic acids or reacting with double bonds.[54] 8-OH Gua is considered an important marker of DNA oxidation.[55] Our findings showed that the ovarian tissues of I/C and I/R/C animals had higher levels of 8-OHGua than those of SSG animals. Nevertheless, our results showed that there were no significant difference between SSG and nanocurcumin-treated animals regarding the levels of DNA damage.

There are many studies in the literature about the improvement of ischemia reperfusion injury. Studies demonstrated that the agents with antioxidant or anti-inflammatory activities may be beneficial in reducing ovarian ischemia reperfusion injury. Also, studies revealed the beneficial effect of controlled reperfusion in the prevention of ovarian tissue damage. In spite of the profuse literature, ischemia/reperfusion damage continues to be a serious problem clinically. Essentially, early diagnosis and treatment of ovarian torsion plays an important role to provide urgent protection against life-threatening complications from ischemia and to prevent future infertility.[56]

Curcumin has been reported as a useful agent both for the prevention and treatment of I/R injury in many organs.[57] These protective effects are mainly believed to be based on inhibitory actions of curcumin on disease-mediated induction of inflammatory transcription factors, protein kinases, adhesion molecules, oxidative stress and inflammation.[57] The administration of curcumin has reported to reduce the generation of reactive oxygen species (ROS), monocyte adhesion, phosphorylation of c-Jun N-terminal kinase (JNK), p38 MAP kinase, and signal transducer and activator of transcription (STAT)-3 in TNF-a-stimulated cells.[57] It has also been documented that the administration of curcumin prior to conservative surgery (detorsion) provides a significant decrease in the oxidative stress markers in the ovarian tissues.[21] The comparison between oxidative status and antioxidative status is clear enough to suggest that the administration of curcumin, as reported previously, leads to a decrease in the oxidative stress and an increase in antioxidation.[21]

Nano-sized particles ranging below several 10 nm are of great interest, because of the chemical and physical behavior of the particles arising from a quantum size effect, which is remarkably different from those in bulk and provides a great potential for use in practice.[58] The findings of the present study showed that nanocurcumin at very low concentrations, 1 mg/kg nanocurcumin versus 100 mg curcumin, produced significant improvements compared to native curcumin.

Substances are administered by a wide variety of routes. A key factor determining the route selected is whether the agent is being administered for a local or systemic (either enteral or parenteral) effect. Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism, which occurs commonly with orally-administered drugs.[58] Intraperitoneal administration seems more effective and may increase drug availability if oral administration poses any difficulties. It is clear that transperitoneal absorption of the agent is much faster than oral administration.[25] Timely treatment is very important in emergency conditions such as ovarian torsion.

In conclusion, histopathological results obtained from all the experimental groups were consistent with the results of the biochemical analyses indicating that intraperitoneal administration of nanocurcumin can be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia. Regarding the transperitoneal absorption of nanocurcumin, which is much faster than its oral administration, transperitoneal ad-

ministration of nanocurcumin seems useful when ovarian torsion takes place. This may help the patients preserve their future fertility. Our study demonstrated that intraperitoneal administration of 1 mg/kg nanocurcumin can improve ischemia-reperfusion injury in ovarian tissue exposed to ischemia. Thus, dose-response studies should be conducted for nanocurcumin to determine its maximal efficacy in minimizing ischemia-reperfusion injury in ovarian tissue.

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Mental status and suicide probability of young people: A cross-sectional study

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Summary

Objective: The most important determinant of suicide ideation, tendency and initiative is the presence of mental disorders. Since the number of those who lost their lives due to suicide in the world rose rapidly among the young population, the World Health Organization emphasizes the importance of assessing young people in the high-risk age group to prevent suicidal behavior. This study aimed to determine psychological symptom levels and suicide probability in young people.
Method: The cross-sectional research consisted of 15-24 year-old individuals (N=348), who have sought a psychiatric clinic between February and June, 2015. The Research Data was collected by applying Data Collection Form, Suicide Probability Scale (SPS) and Brief Symptom Inventory (BSI). SPSS 22.0 statistical package program was used for data analysis.
Results: There was a statistically significant difference ($p<0.05$) between the mean SPS scores according to education, psychiatric treatment, self-harm, smoking and drinking status of the participants in the study. Apart from this, there was also a statistically significant correlation between anxiety, depression, negative self and hostility according to the SPS and BSI subscales ($p<0.001$, $r=0.739$; $p<0.001$, $r=0.729$; $p<0.001$, $r=0.747$; $p<0.001$, $r=0.715$; respectively).
Conclusion: The results of our study show that suicide risk is significantly higher in young people with depression, anxiety, negative self-perception and hostility symptoms. In this regard, we suggest the relevance of assessing the suicide risk of young people seeking a psychiatric clinic, with thorough attention to those who have high potential for suicide.

Keywords: Anxiety. Depression. Hostility. Suicide. Young Adult. Adolescent.

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Introduction

Suicide is a multifaceted behavior that occurs as a result of many psychological, sociological, economic and cultural factors. Therefore, suicide rates have been reported to be associated with physical, biological and mental health variables, as well as variables such as the country's lifestyle, religious tendencies, social class, age, gender, education, marital status.[1,2]

The World Health Organization (WHO) reports that around one million people die due to suicide each year in the world and the suicide rate increases in the young age group.[3] It has also been reported that the worldwide annual suicide rate is 16 per 100,000, which has increased by 60% in the last 45 years.[4,5] In Turkey, the number of suicide deaths in 2013 is 4.19 per 100,000. It is reported that the highest figures in these deaths belong to the young population between the ages of 15-19 years and the suicide cases in the age range of 15-24 are reported to be high.[6] In a survey conducted by the Turkish Statistical Institute in Izmir, it was determined that, in 2012, 44.9% of all suicide attempts occurred in young people between 15-24 years of age.[7]

Young people apply various lethal methods with the aim of suicide. These methods include firearms, drug intake, self-suffocation, burning, stabbing, jumping to traffic, drowning in water. It has been reported that the most common method of attempting suicide among adolescents is deliberately overdosing on drugs. Further-

more, it is known that suicide attempts among women are more frequent and that women apply less lethal methods than men.[8,9]

The most important determinants of suicide ideation, suicidality and suicide attempt are the presence of mental disorders.[10] It has been determined that more than 90% of people who have attempted suicide have at least one psychiatric disorder, according to various researches.[11,12] One of the largest epidemiological studies performed in our country, the Turkey Mental Health Profile Study, revealed that 18% of the Turkish population have a mental disorder during their lifetime. Suicide rates related to these mental illnesses have been considered very high.[13]

As the number of people losing their lives due to suicide increases rapidly, it has to be accepted as an important public health problem both in the world and in our country.[14] The WHO emphasizes the importance of evaluating young people who are in high risk groups (15-24 years) and of performing studies (early diagnosis and treatment, planning of preventive mental health services, elimination of lack of knowledge on the subject etc.) to prevent suicidal behavior.[14,15] This duty and responsibility belongs to the health professionals who are working with young people. With the suicide prevention work performed by professionals, the risk factors will be reduced, protective factors will be strengthened and thus reveal the healthy behaviors of youngsters.[16,17]

After a literature review, it has been determined that there are insufficient studies analyzing the relation between mental symptoms and suicide variables and that problematic behaviors seen during one's youth increase the rates of suicide. Considering that young individuals who have sought a psychiatric clinic due to mental problems comprise a risk group, our study aimed to analyze the mental symptom levels of these patients and to prevent suicide by determining preventive and protective mental health studies accordingly. Additionally, we aimed to determine the psychological symptoms and probability of suicide according to their perception.

Method

This is a cross-sectional study that included young individuals aged 15-24 years who sought the psychiatry outpatient clinic of the Ankara Education and Research Hospital due to mental problems. The research was conducted between February and June 2015. 348 consecutive patients, 29.9% (104) of males and 70.1% (n = 244) of females between 15-24 years, who sought our psychiatric outpatient clinic comprised our sample. They all had primary school education and no problems of speech, understanding and communication. In the power analysis conducted to determine the adequacy of the sample volume, the power of the study was set at 80% with a confidence level of 95% and a significance level of 0.05. These figures indicate that the sample volume is sufficient.

Research data were collected by applying a Data Collection Form, Suicide Probability Scale (SPS) and Brief Symptom Inventory (BSI). This was carried out by filling the forms during 20 minutes of face-to-face interviews under the supervision of the researchers.

The Data Collection Form consisted of questions establishing individual traits of the participants (age, gender, education level, family support, family type, economic status, receiving psychiatric treatment etc.).

The Suicide Probability Scale (SPS) is a Likert-type scale, which has been developed by John G. Cull and Wayne S. Gill (1990) in order to evaluate suicide probability and consists of 36 articles for self-assessment (self-report). It is used on adolescents and adults to evaluate suicide risk. Scale adaptation, reliability and validity studies by the Turkish Society were performed for the first time by Tugcu (1996), while validity and reliability studies on the Atlı clinic sampling were performed in in 2007. It was identified that the internal coefficient of consistence of analyzed scale for "total point" is .87, test re-test reliability is .98, similar scale validity is .84. The sum of all scores in the scale yields a general suicide probability. High scores indicate that the probability of suicide is high.[16,18]

The Brief Symptom Inventory (BSI) is a likert-type self-assessment scale that scans psychological symptoms such as anxiety (articles 12, 13, 28, 31, 32, 36, 38, 42, 43, 45, 46, 47 and 49), depression (articles 9, 14, 16, 17, 18, 19, 20, 25, 27, 35, 37 and 39), negative self-perception (articles 15, 21, 22, 24, 26, 34, 44, 48, 50, 51, 52 and 53) somatization (articles 2, 5, 7, 8, 11, 23, 29, 30, and 33) and hostility (articles 1, 3, 4, 6, 10, 40 and 41); it consists of five subscales and 53 articles. The point range is between 0-212. The scale is intended for adolescents and adults, individually or as a group. There is no time limit to answer the scale. The height of the total points obtained from the scale indicates the frequency of symptoms of the individual.[19,20] The validity and reliability for adolescents have been established by Sahin et al. and the internal coefficient of consistence of subscales has been found as follows: .70 (somatization) and .88 (depression), while the coefficient of internal consistency of the inventory was identified as .94.[19]

Ethical aspect of the research

Before starting the research, the institution's approval (03.12.2014 / 0572) was obtained. This study was carried

out after having obtained Ankara Training and Research Hospital Ethics Committee (08.01.2015 / E-15-317) approval. Additionally, the adolescents voluntarily participating were informed about the purpose of the study and their written consent was obtained.

Data evaluation

Statistical Package for the Social Sciences (SPSS software version 22.0, SPSS Inc., Chicago, IL, USA, undergraduate, Hitit University) was used for data analysis. Number and percentage values are given in the distribution of findings on the individual characteristics of the participants. Continuous data are presented as mean ± standard deviation. Normal distribution was examined by Shapiro Wilks normality test. Based on individual characteristics, the participants were divided in two independent groups and compared by means of Student's t-test and Kruskall-Wallis test results. Spearman's rho correlation coefficient and linear regression analysis were used to investigate the relations between SPS and KSE subscales, which was accepted as $p<0.05$ for statistical significance.

Limitations of the study

This research was carried out to determine the psychological indications and suicide probabilities of young people aged 15-24 years and is limited to the data obtained from 348 young people who have sought the psychiatric clinic between February and June 2015. In addition, the research data for determining suicide probabilities and the psychological symptoms of the participants are limited to the answers given by them.

Results

A total of 348 young individuals participated in the survey, 70.1% were female and 29.9%, male. The mean age of the participats was 21.04±15.02 years and their distribution according to individual characteristics is given in Table 1.

It has been reported that the total SPS mean point of young people participating in the study is 77.52±13.21 (Min: 43; Max: 113). An examination of the relation between SPS scores according to some characteristics of the adolescents is given in Table 1 and there is a statistically significant difference between SPS mean scores according to education, present psychiatric treatment, self-harm, smoking and drinking status ($p<0.05$).

BSI mean score of the surveyed participants has been determined as 78.33±41.47. When the means of the scores of BSI anxiety, depression, negative self-perception, somatization and hostility subscales were examined, the mean depression score was found to be higher than the other subscale scores (Figure 1).

TABLE 1 Comparison of the average scores for Suicide Probability Scale (SPS) according to the individual characteristics of youth (N=348).

Individual characteristics	n	%	Suicide Probability Scale X±SD	Statistical analysis
Age				
15-18	102	29.4	79.23±12.38	p=0.102
19 and older	246	70.6	76.62±13.93	
Gender				
Male	104	29.9	76.90±12.16	p=0.300
Female	244	70.1	77.78±13.68	
Educational level				
Elementary	54	15.5	81.14±17.55	p=0.049*
High school	154	44.3	75.49±12.36	
University	140	40.2	78.35±11.96	
Family support				
Yes	70	20.1	77.41±12.66	p=0.677
No	278	79.9	77.94±15.42	
Family type				
Nuclear	262	75.3	78.00±12.84	p=0.654
Extended	44	12.6	76.68±13.40	
Broken	42	12.1	81.28±12.29	

(*Continues*)

TABLE 1 (Cont.) Comparison of the average scores for Suicide Probability Scale (SPS) according to the individual characteristics of youth (N=348).

Individual characteristics	n	%	Suicide Probability Scale X±SD	Statistical analysis
Family income status				
Income lower than expenses	98	28.2	75.97±12.67	p=0.588
Income equal to expenses	206	59.2	76.94±12.70	
Income higher than expenses	44	12.6	83.68±15.54	
Received currently psychiatric treatment				
Yes	154	44.3	79.71±15.08	p=0.012*
No	194	55.7	75.78±11.30	
Bodily harmed him or herself				
Yes	144	41.4	82.88±14.83	p=0.008**
No	204	58.6	73.50±11.09	
Smoking habit				
Yes	230	66.1	80.23±12.26	p<0.001***
No	118	33.9	72.23±13.50	
Alcohol drinking habit				
Yes	166	52.3	79.48±12.80	p=0.048*
No	182	47.7	75.37±13.40	
Total	348	100	77.52±13.21	

Note. Values are given either as %, * p<0.05, ** p<0.01, ***p<0.001, arithmetic mean (X) ± standard deviation (SD).

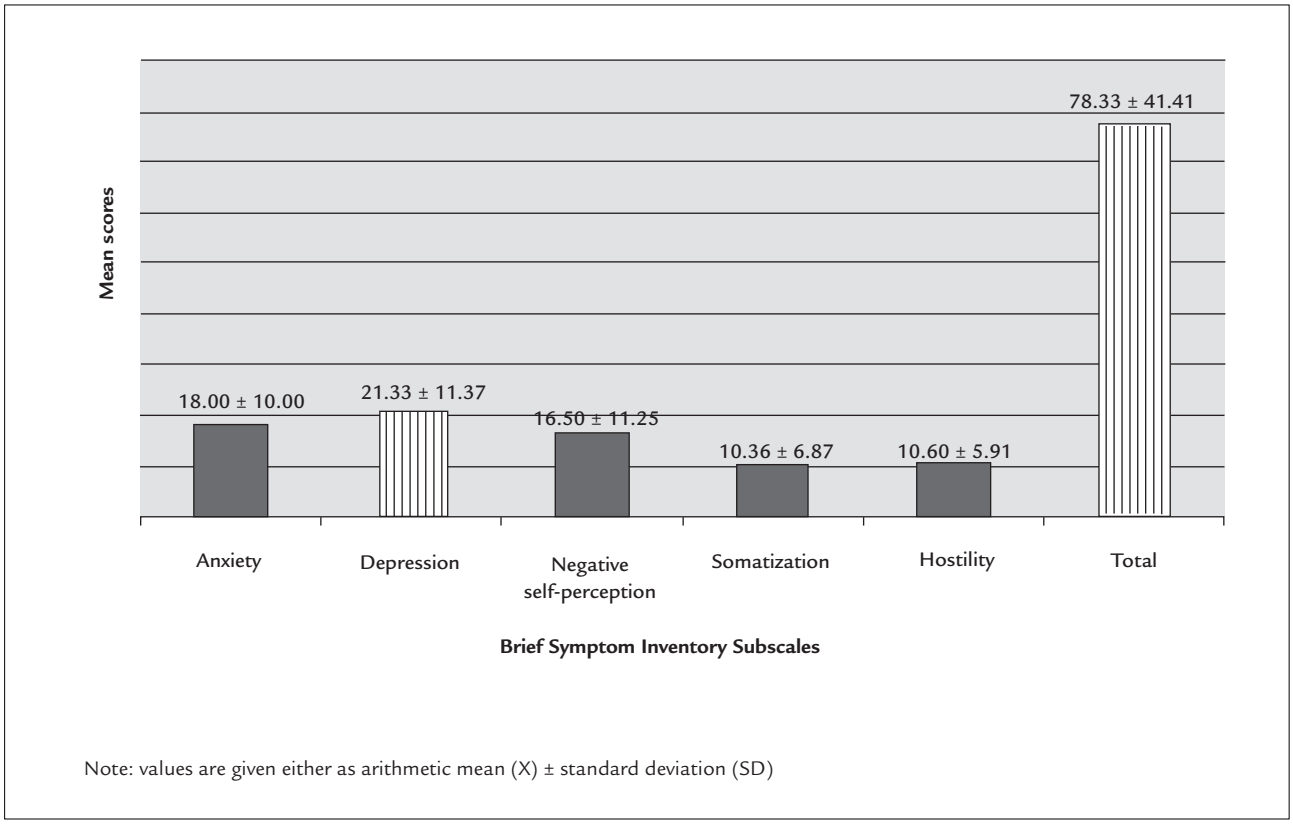

FIGURE 1 Distribution of Youth' Brief Symptom Inventory Subscales mean scores.

After investigating which of the BSI subscales had a greater effect on the SPS subscales according to the results of correlation analysis, there was a statistically significant correlation between anxiety, depression, negative self and hostility ($p<0.001$, $r=0.739$; $p<0.001$, $r=0.729$; $p<0.001$, $r=0.747$; $p<0.001$, $r=0.715$; respectively), as well as a significant moderate correlation with somatization ($p<0.001$, $r=0.582$, Table 2). In the single variable regression model, the BSI subscales of anxiety, depression, negative self, somatization and hostility variables of the explanatory coefficients were $R2=0.537$, $R2=0.510$, $R2=0.538$, $R2=0.346$, $R2=0.501$, respectively (Figure 2).

Discussion

According to the WHO and the Turkish Statistical Institute data, suicidal behavior has been increasing in many countries over the years and is now considered a universal problem. Psychiatric disorders are seen as the most common cause of suicide.[6,12,21] Our study, which evaluates the possibility of suicide in the 15-24 age group seeking our psychiatry clinic, is important as it draws attention to the need of suicide risk assessment and risk management in psychiatric clinics within the scope of patient safety.

We observed that 29.4% of the participants who sought our psychiatry clinic were in the age range of 15-18 years and 29.9% were male (Table 1). It has been reported that male adolescents have a slightly higher rate of admission to psychiatry clinics during early adolescence, this ratio is equal for both genders in mid-adolescence, and a significant increase is observed in young adulthood in girls.[22] Our findings support the literature if we consider that the young people participating in our study are among the 15-24 age group and 70.1% are girls.

The United Nations International Children's Emergency Fund (UNICEF) analyzed the Situation of Child and Youth Population in Turkey in 2012 and 36% of the youth between the ages of 15-24 years were in full-time general or vocational education in 2011; 32% of the young population were employed; and 32% were not either studying or working.[23] In our study, 44.3% of the youth are high school graduates and 79.9% are not receiving family support (Table 1). The fact that the vast majority of our study's participants have a high school diploma and do not receive financial support from their families suggests that this specific population works more hours than the mean for this age group in Turkey.

Drug use is seen as a major social problem. The number of substance addicts increases every day, causing the problem to grow more and more. Measures to prevent drug addiction are also being attempted in our country, where the young population is large and efforts are being made to prevent the spread of substance use.[24] According to the Global Adult Tobacco Survey Turkey Report conducted in our country in 2012, it has been reported that the frequency of smoking among those aged 15 years and older in our country is 27.1% and that this rate is 8.4% in young people.[25] Ozcebe reported that there is a 33.2% prevalence of cigarette smoking among young people and the progression from school to the labor market is affecting smoking behavior.[26] The result of our study shows that the vast majority of young people who seek psychiatric clinics do not receive family support, 66.1% smoke and 52.3% use alcohol (Table 1), which is in line with the literature. Although prevention has been portrayed as the most important step against smoking and alcohol use among the young, active interventions targeting current users are also extremely important.

According to the literature, the majority of individuals who have suicide ideation or attempt suicide are between 15-24 years old.[27,28] While the ratio of those who attempted or succeeded in committing suicide in Turkey is lower than in European countries, the majority of young people who have attempted suicide are in the age range of 15-19 years.[4,6] The probability of suicide found in our study was higher in the 15-18 age range than in those aged 19 years or more (Table 1). Our result supports the literature.

Mean SPS score of women in our study (77.78±13.68) has been found to be higher than that of men (76.90±12.16), although no statistically significant difference has been identified regarding gender and suicide probability ($p>0.05$; Table 1).While in the study conducted by Langhinrichsen-

TABLE 2 Correlation coefficients of youth' Suicide Probability Scale and Brief Symptom Inventory Subscales.

Suicide Probability Scale	Brief Symptom Inventory Subscales				
	Anxiety	Depression	Negative self-perception	Somatization	Hostility
r	0.739	0.729	0.747	0.582	0.715
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Note. *Spearman's correlations statistically significant, $p<0.001$. Correlation is statistically significant ($0.00 < r < 0.25$: little if any correlation; $0.26 < r < 0.49$: low correlation; $0.50 < r < 0.69$: moderate correlation; $0.70 < r < 0.89$: high correlation; $0.90 < r < 1.00$: very high correlation).

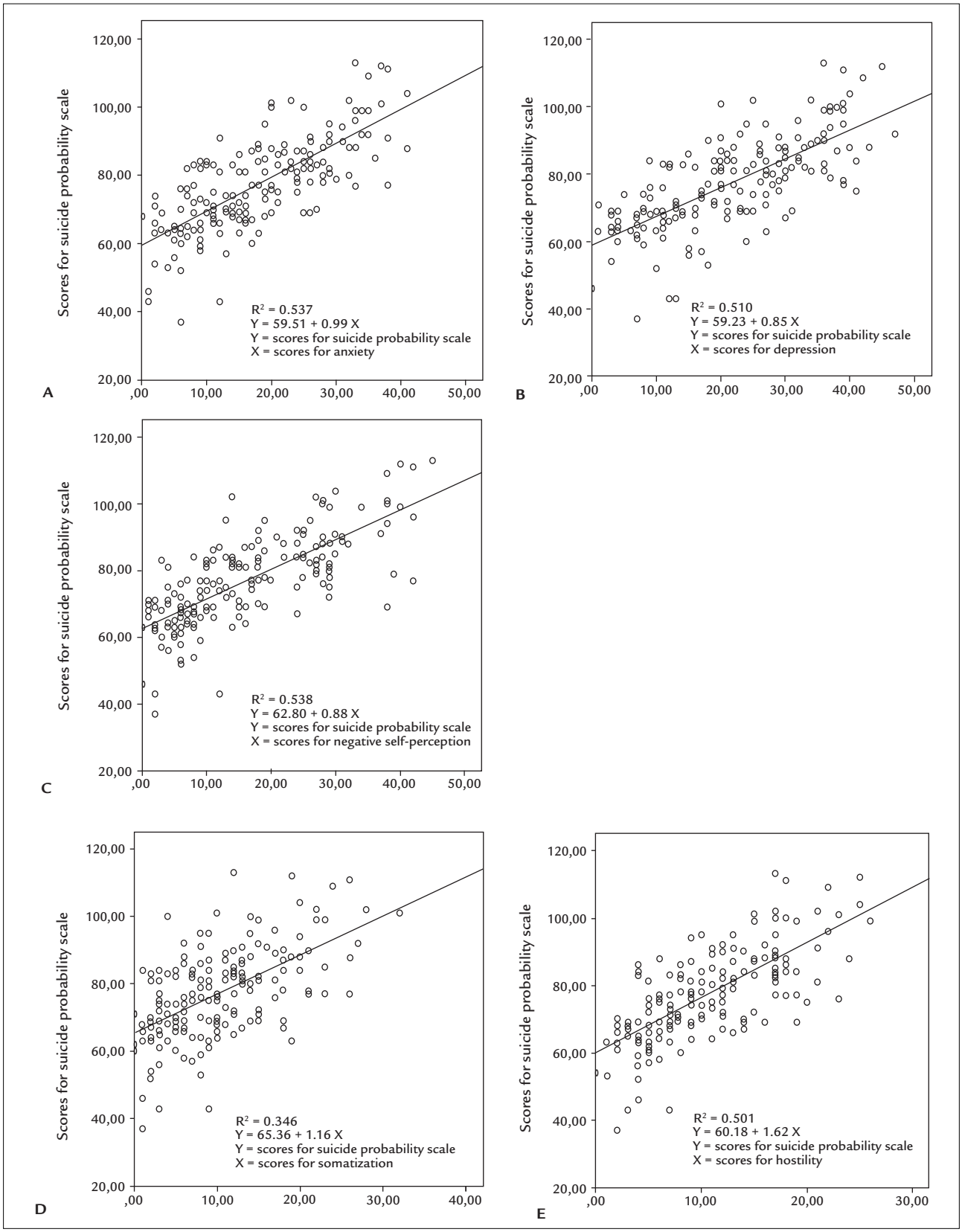

FIGURE 2 Regression analysis for scores of Suicide Probability Scale and Brief Symptom Inventory Subscale; scores for (A) anxiety, (B) depression, (C) negative self-perception, (D) somatization, (E) hostility.

Rohling et al.[29] no difference could be found between genders regarding suicide ideation, Molina and Duarte[30] reported that the number of women in their study was higher than that of men. However, Whetstone et al.[31] determined that men have more suicide ideation than women.[29-31] In various studies carried out in Turkey, it has been identified that the number of women who have attempted suicide is higher than that of men.[16,32] This may be caused by the manner women are traditionally perceived and respond to social convention in Turkey.

The literature indicates that the more the education level decreases, the more suicide rates increase. The most important determinant for increasing the suicide rate has been identified as low education level. It is thought that the ability to think of people with suicide risk weakens due to a sense of helplessness, hopelessness and stress, and that their problem-solving skills are reduced.[33,34] The suicide rate of university graduates is low, according to the statistics and studies carried out in Turkey.[6] While the high suicide probability of primary school graduates in our study supports the literature, the reasons for higher suicide probability of university graduates compared to high school graduates have to be examined.

In our study, it was determined that those who do not receive family support, live with a single parent or belong to a nuclear family, and have lower income than expenditures show a higher suicide risk compared to those who receive family support, have a big family and have an income equal to or higher than their expenditures (Table 1). According to the literature, the majority of individuals who have attempted suicide come from a broken family and present a bad economic income level; it has been reported that this situation is a factor that effects suicidal behavior.[34,35] Furthermore, studies[35,36] reported that suicide attempts are frequently seen in nuclear family members and as a reason for that the inadequate support system compared to the support system in extended families has been asserted. Interfamilial support in traditional family structure is very strong, thus strengthening the individual's coping mechanisms and reducing the frequency of suicide attempts. Kleiman and Liu[37] noticed that lifelong social support is related with the decrease of suicide attempt probability and therefore suggest that this can be used as a factor in developing the current suicide prevention programs worldwide.[37]

According to the literature, 90-95% of those who have put an end to his own life or attempted suicide have at least one mental illness and the suicide rate in all psychiatric patients was 3-12 times higher than in the normal population.[11,16] In our study, we report that the suicide rate of those (79.71±15.08) who receive psychiatric treatment is higher than that of those who do not receive treatment (75.78±11.30) (Table 1). The findings of our study support the view that psychiatric treatment has a direct effect on suicide ideation and reveal the importance that professionals working with young people have in being more cautious with those who receive psychiatric treatment in terms of suicide probability.

In our study, it was determined that young people who have ever bodily harmed themselves have a higher probability of suicide (Table 1). In line with our findings, it is mentioned in the literature that people with physical self-injurious behavior are more likely to commit suicide.[13,38] Additionally, the view of a strong relationship between physical self-harm and suicide probability is supported by the literature. Hawton and James[39] reported that self-injurious behavior in adolescents is often impulsive and that they think for only a few minutes before taking action.[39] As a result of this suicidal behavior, the action can be serious and life-threatening even if there is no apparent wish to die. Therefore, all kinds of attempt, even if there is no apparent wish to die, should be handled very seriously because of the suicide probability.

It has been noticed that the majority of adolescents in our study smoke (66.1%) and drink alcohol (52.3%). It has been identified that young individuas who smoke and drink alcohol show a higher suicide probability compared to those who do not smoke and drink and this difference has been found statistically significant ($p<0.001$ and $p<0.05$, respectively, Table 1). Our finding is compatible with the fact that suicide ideation and attempts are mostly seen combined with cigarette and alcohol consumption, as in studies mentioned in the literature.[32,40] Deveci et al.[32] reported that cigarette and alcohol consumption is statistically frequently seen in individuals who have suicide ideation while high cigarette consumption is related to suicide attempts.[32] Carballo et al.[40] reported that adolescents who consume alcohol tend more to suicide when they show depression or anxiety disorder symptoms.[40]

When the BSI subscales used for psychiatric evaluation in our study were evaluated, the depression score was the highest and somatization was the lowest (Figure 1). Similarly to our results, Tanrıverdi and Ekinci[41] also found the highest means for depression and the lowest for somatization. Findings differ in various studies. While in the study of Yıldırım et al.[42] the highest mean was attributed to somatization, depression was the second highest. According to Barlas et al.,[43] the highest mean refers to anxiety and the second highest, to depression. These results suggest that young people experience different mental problems and are especially at risk for depression and anxiety.

In our study, according to the BSI subscale and SPS score averages reached regression equation as a result of regression analysis, 1 unit increased anxiety 0.99, depression 0.85, negative self 0.88, somatization 1.16, and hostility 1.62 increased the suicide score (Figure 2). We also determined that the probability of suicide was close to that of all diagnostic groups when the total SPS score average was evaluated according to the diagnostic groups of the participants, with a risk of suicide significantly higher in young people with depression, anxiety, negative self-esteem and hostility symptoms (Figure 2 and Table 2). Similar to our findings, the literature emphasizes that people with mental problems should be carefully evaluated for the possibility of suicide.[44,45]

Based on these results, we recommend that a top priority suicide risk assessment should be performed on adolescents who seek psychiatric clinics, who have high suicide potential and indication of suicidal intent. In addition, health professionals working with young people should be made aware of risk management and improved risk management understanding.

Conflict of interest

The authors declare no conflict of interest.

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Analyzing the neuropsychological characteristics and changes in serum markers of patients with chronic cerebral circulation insufficiency

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Summary

Objective: To investigate the neuropsychological characteristics and changes in CRP, S100B, MBP, HSP-7, and NSE in serum.
Method: Sixty-six (66) patients treated in our hospital as CCCI group were chosen for our study, and 90 patients with depression were selected as the depression group. The patients in both groups were examined with CT perfusion, depression, anxiety and cognition evaluation. Their serum CRP, S100B, MBP, HSP-70 and NSE levels were detected. Neuropsychological and serum markers characteristics were compared.
Results: The CBF and CBV in bilateral basal ganglia, frontal lobes, greater oval center, brain stem, and left and right regions of occipital lobes of the patients in CCCI group were significantly lower than in the depression group. The HAMD and HAMA scores of CCCI group patients were significantly lower than in the depression group; CCCI group performed better regarding attention, memory, abstract terms and delayed recall. CCCI also had significantly higher total scores than the depression group. Serum CRP, S100B, MBP, HSP-70 and NSE levels in CCCI group were significantly higher than in the depression group. The differences reach statistical significance ($p<0.05$).
Conclusion: CCCI patients who are accompanied by minor depressive disorder have different degrees of cognitive impairment and experience a significant rise in serum CRP, S100B, MBP, HSP-70 and NSE.

Keywords: Neuropsychology. Biomarkers. Cerebrovascular Circulation.

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Introduction

Chronic cerebral circulation insufficiency (CCCI) is cerebral vascular stenosis or hypoperfusion in patients induced by multiple factors. It can cause the cerebral blood flow to be incapable of satisfying the basic physiological demands.[1-3] Its existence has been one of the most controversial issues in this field because this disease has no specific clinical manifestations and there is no reliable diagnosis method for it.[4-6] However, a large number of clinical experiences have demonstrated that such patients often experience the clinical manifestations of repeated dizziness and head heaviness, which are accompanied by emotional abnormalities such as anxiety-depression of varying degrees, with a very high possibility of acute stroke and cognitive impairment. During recent years, in order to find an accurate method to diagnose CCCI, the changes in the level of serum markers have drawn wide attention from scholars.[7,8] Therefore, our study reviewed the CCCI patients admitted in our hospital from April 2013 to April 2015 and analyzed their psychological characteristics as well as serum markers.

Method

General materials

A total of 66 CCCI patients visited our hospital for treatment from April 2013 to April 2015, meeting the diagnosis criteria: (1) aged over 60 years; (2) with the risk factors of cerebrovascular diseases; (3) with a course of chronic diseases for over half a year; (4) with subjective symptoms such as obvious dizziness and head heaviness; (5) cerebral arterial vascular stenosis displayed by MRA/DSA examinations. In the CCCI group, the number of male and female patients was 42 and 24, respectively, aged 60 to 79 years with a mean of 68.31±5.24 years. Additionally, 55-year old individuals

with depression who sought our hospital during the same period were selected and included as a control group, with the clinical manifestations of similar dizziness and vertigo. The control group had 25 males and 20 females aged 61 to 78 years with a mean of 67.32±4.27 years.

Study methods

CT spiral perfusion examination: all patients underwent cerebral CT perfusion imaging examination within one week after their admissions. The examination was performed with a 64-layer spiral CT machine (Brilliance from Phillips Company). Using the canthomeatal line as the baseline, the conventional cross section scan with a layer thickness of 5 mm and a layer spacing of 5 mm was first performed, and then CT perfusion imaging was performed with basal ganglia plane as the center and a coverage area of 40 mm (layer thickness 5 mm × 8 layers). By means of high-pressure syringe, a bolus injection of 50 mL non-ion contrast medium (ultravist 300 mg/mL) was performed at the rate of 4 mm/s via the hand dorsum vein. In the meantime, dynamic scan (matrix 512 × 512, scan field 24 × 24 mm, tube voltage 120 kV, tube current 80 mA) was performed continuously for 50 seconds, the re-acquired 152 dynamic images were transmitted to the workstation, and Brain perfusion software was used for further processing. The planes at the basal ganglia and corona radiata areas of the CCCI patients were selected as the planes of interest, and a method of hand drawing of the region of interest (ROI) was adopted to observe the following regions: bilateral basal ganglia, frontal lobes, greater oval center, brain stem and occipital lobes. Parameters such as cerebral blood flow, cerebral blood volume, mean transit time (MTT) and time-to-peak (TTP) were measured. Generally, each site was measured for three times to ensure that the size of ROI selected each time was reasonably consistent. Both qualitative and quantitative methods were adopted to evaluate the computed tomography perfusion imaging (CTPI) parameters, i.e. a qualitative evaluation on whether the left and right cerebral perfusion was symmetric on the TTP and MTT pseudocolor images: with the measured values in the control group as the yardstick, if the absolute value of CTPI parameters of each ROI in CCCI group exceeded 95% confidence interval of the absolute values in the control group, that was considered as perfusion abnormality.

In the next morning following admission, 5 mL of venous fasting blood were extracted as detection samples, then they were centrifuged, and finally the supernatant was saved and stored at -80°C. Radioimmune turbidimetric method was used to measure the C-reactive protein (CRP) levels of the patients in the two groups after admission and after the treatment, and the enzyme linked immunosorbent assay was used to determine the S100 calcium-binding protein B (S100B), myelin basic protein (MBP), heat shock protein 70 (HSP-70) and neuron-specific enolase (NSE) contents in plasma.

Grade indexes

Status evaluation: the Hamilton's Depression Scale (HAMD) and Anxiety Scale (HAMA) were used to evaluate the patient's depression and anxiety status after admission and after treatment.

Cognitive function evaluation: the Montreal Cognitive Assessment (MoCA) scale was used to determine the patient's cognitive functions after admission and after treatment, the scale is mainly used to explore eight aspects of cognitive functions, such as visual space, executive capacity, naming, ability to concentrate mentally and language. The test generally lasts 8 to 10 minutes. A higher score means stronger cognitive abilities.

Statistical methods

SPSS 15 software was used for data processing, the enumeration data were shown in the form of absolute numbers and frequencies, t and χ^2 methods were used for the test. $p<0.05$ means the difference was of statistical significance.

Results

CT perfusion parameters in the left and right encephalic regions

As is shown in Figure 1, the cerebral blood flow (CBF) and cerebral blood volume (CBV) in bilateral basal ganglia, frontal lobes, greater oval center, brain stem, and left and right regions of occipital lobes of the patients in CCCI group were significantly lower than those seen in the depression group. The comparison generated a significant difference ($p<0.05$ or $p<0.01$), and the inter-group comparison did not generate an obvious difference in MTT and TTP ($p>0.05$).

Depression and anxiety evaluation

As shown in Table 1, the HAMD and HAMA scores of patients in the CCCI group were significantly lower than those of the depression group, and the comparison generated a significant difference ($p<0.05$). The proportions of patients with possible depression and anxiety in the CCCI group were significantly higher than that of the depression group, but the proportion of patients with confirmed depression and anxiety and obvious depression and anxiety was significantly lower than that of depression group, and the comparison generated a significant difference ($p<0.05$).

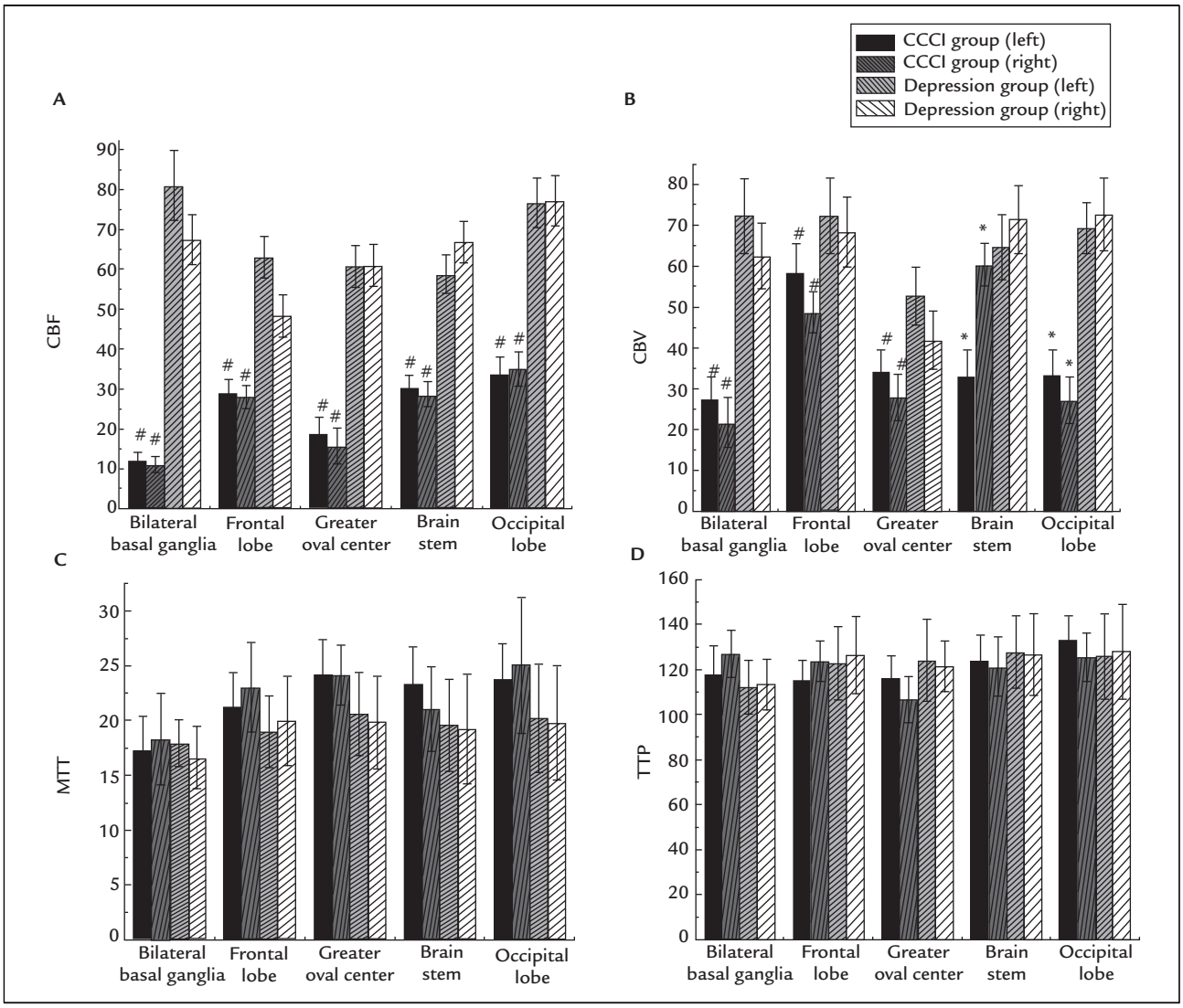

FIGURE 1 Comparison between CCCI group and depression group in terms of the patient's perfusion parameters of left and right encephalic regions when compared with the depression group. *p<0.05; #p<0.01.

CCCI: chronic cerebral circulation insufficiency; CBF: cerebral blood flow; CBV: cerebral blood volume; MTT: mean transit time; TTP: time-to-peak.

TABLE 1 Comparison between CCCI group and depression group in terms of anxiety-depression.

Group	Depression					Anxiety				
	HAMD score	Normal	Possible depression	Confirmed depression	Serious depression	HAMA score	No anxiety	Possible anxiety	Confirmed anxiety	Obvious anxiety
CCCI group (n=66)	23.22±5.27	8 (12.12)	36 (54.55)	19 (28.79)	3 (4.55)	17.32±4.29	14 (21.21)	32 (48.48)	13 (19.70)	7 (10.61)
Depression group (n=55)	33.21±5.32	0 (0)	10 (18.18)	29 (52.73)	16 (29.09)	26.32±5.21	5 (9.09)	11 (20.00)	26 (47.27)	13 (23.64)
t/χ^2	7.833	-	4.486	5.874	3.76	4.844	4.456	5.477	6.685	4.466
p	0.009	-	0.013	0.017	0.028	0.012	0.015	0.013	0.024	0.034

CCCI: chronic cerebral circulation insufficiency; HAMD: Hamilton's Depression Scale; HAMA: Anxiety Scale (HAMA).

Cognitive evaluation

As shown in Table 2, the scores for visual space, executive capacity, ability to focus, memory, abstraction ability, delayed recall and the total scores of the patients in the CCCI group were significantly lower than those in the depression group, and the comparison generated a significant difference ($p<0.05$).

Plasma CRP, S100B, MBP, HSP-70 and NSE levels

As shown in Figure 2, the levels of CRP, S100B, MBP, HSP-70 and NSE in serum of the patients in the CCCI group were significantly higher than those of the depression group, and the comparison generated a significant difference ($p<0.05$).

Discussion

In 1990, a scholar from Japan first proposed the concept of chronic cerebral circulation insufficiency, which refers to a phenomenon of overall blood flow decrease that occurs in the brain instead of the focal ischemic lesions.[5] Studies have revealed that the primary reason for CCCI occurrence is as follows: atherosclerosis leads to the occurrence of vascular plaques and stenosis, so that the cerebral blood flow decreases. When it decreases to a certain threshold value, the local perfusion will also decrease, causing clinical manifestations such as dizziness and head heaviness, which are usually considered to be the early manifestations of cerebral infarction.[9-12] As there is no specific manifestation for CCCI clinical symptoms and imaging characteristics, early accurate diagnosis is one of the difficulties in the treatment of this disease. Currently, domestic and foreign studies show that the early manifestations of many patients with chronic cerebral functional insufficiency are experiencing an increase in memory loss and emotional disorders, which is similar to neurosis such as depression and accompanied by mild cognitive disorder. Therefore, the studies on the neuropsychological characteristics of CCCI patients have drawn wide attention from scholars during the recent years.[13]

The results of our study revealed that 87.88% of the patients in the CCCI group have anxiety issues and 78.78% of the patients concomitantly suffer from the psychological conditions of depression. Comparison between the CCCI group and the depression patients shows that the proportion of patients with possible depression and anxiety in the CCCI group was significantly higher than that of the depression group, but the proportion of patients with confirmed depression and anxiety and obvious depression and anxiety was significantly lower than that of the depression group. It is also found that the HAMD and HAMA scores of the patients in the CCCI group were significantly lower than those seen in the depression group. The results indicated that the majority of the CCCI patients concomitantly suffer from depression, but the depression is mild and there is no specific report on the incidence rate of depression among CCCI patients. Secondly, in our study, the MoCA scale was used to evaluate the cognitive functions of the patients in the two groups. The results indicated that the scores for visual space, executive capacity, ability to focus attention, memory, abstraction and delayed recall and the total scores of the patients in the CCCI group were significantly lower than those in the depression group. The result also indicated that the cognitive functions of the patients in the CCCI patients decreased significantly. The decrease in the cognitive functions of the patients in the CCCI patients has drawn wide attention. Animal experiment studies showed that chronic cerebral ischemia will cause serious damages to the neurons of hippocampal CA1 area of rats while the hippocampal neurons are the key links that influence the cognitive abilities such as memory and learning, which can properly explain the decrease in the cognitive functions of the

TABLE 2 Comparison between CCCI group and depression group in terms of MoCA score.

Item	Full score	CCCI group (n=66)	Depression group (n=50)	t	p
Visual space and executive capacity	5	4.39±0.26	4.54±0.31	5.967	0.028
Naming	3	2.23±0.17	2.41±0.27	0.876	0.142
Ability to concentrate mentally and memory	6	4.34±1.01	5.12±0.57	2.837	0.037
Language	3	2.42±0.31	2.68±0.18	0.412	0.052
Abstraction	2	1.17±0.25	1.52±0.17	3.076	0.026
Delayed recall	5	2.57±0.83	3.78±0.54	6.983	0.017
Orientation	6	5.73±0.11	5.76±0.07	0.928	0.219
Total score	30	22.27±3.56	25.27±3.13	4.976	0.026

CCCI: chronic cerebral circulation insufficiency; MoCA score: Montreal Cognitive Assessment score.

CCCI patients. Studies revealed that basal nuclei areas and greater oval centers are rich in a large number of neurons and fibers closely associated with cognitive functions such as learning and memory.[14-17] By means of multi-layer spiral CT examination, our study found that the CBF and CBV in bilateral basal ganglia, frontal lobes, greater oval center, brain stem, and left and right regions of occipital lobes of the patients in CCCI group were significantly lower than those seen in the depression group, which leads to decrease in the cognitive functions of the CCCI patients.

Change in blood markers is also one of the most important manifestations of chronic cerebral ischemic diseases.[18,19] In our study, the research of all indices in blood indicated that CRP, S100B, MBP, HSP-70 and NSE in the blood of patients in the CCCI group had a significant increase compared with the depression group. CRP level is one of the commonest markers for body inflammatory reactions during the clinical application. A large number of studies revealed that increased levels of CRP in plasma of patients with chronic cerebral ischemia are closely as-

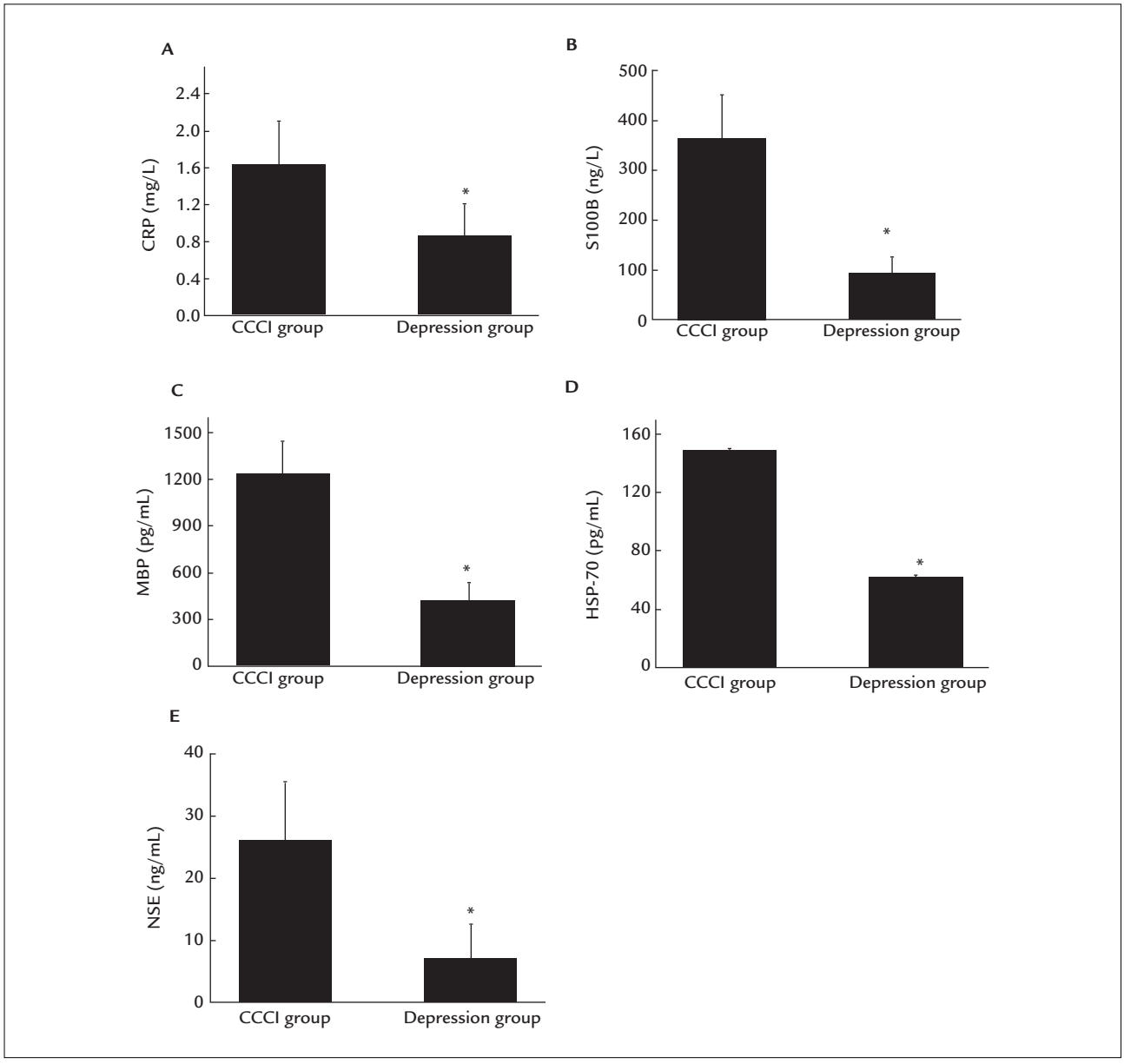

FIGURE 2 Comparison the levels of CRP, S100B, MBP, HSP-70 and NSE between the CCCI group and depression group. Data are presented as mean + SD. *p<0.05 versus CCCI group.

CCCI: chronic cerebral circulation insufficiency; CRP: C-reactive protein; MBP: myelin basic protein; HSP-70: heat shock protein 70; NSE: neuron-specific enolase.

sociated with the degree of injuries in brain cells. And it can further aggravate the injuries of cerebral vascular endothelial cells and aggravate patient conditions.[20] NSE is a type of specific enolase present in the cerebral neurons and endocrine cells and participates in the glycolysis process. Cerebral blood supply insufficiency will lead to functional disorder and structural injuries in neuron serous membrane. As a result, NSE will be released from the damaged cell membrane and enter the cerebrospinal fluid, passing through the disrupted blood-brain barrier and entering the blood circulation, further leading to an increase in the level of NSE in blood. S100B and MBP are present in the colloid and oligodendrocytes in the central nervous system. Their abundance can somewhat reflect the degree of damages of colloid and oligodendrocytes and is closely associated with the damages in cognitive functions.[21] NSE, S100B and MBP are often used to evaluate whether the damages occur to neurons, colloid and oligodendrocytes in the cerebral tissues. They can also be used to evaluate severity because they are important serum markers of cognitive function. Studies revealed that HSP-70 is a type of stress protein closely associated with patient cognitive functions, and HSP-70 can improve neurocyte tolerance and activate the anti-apoptosis route under the stress status so that the damaged neurocytes in the brain can be protected. Therefore, in the case of cerebral blood supply insufficiency in the human body, the HSP-20 level will be greatly increased to lower the neurocyte injuries.[17] As shown in the above results, in the case of the occurrence of CCCI, the CRP, S100B, MBP, HSP-70 and NSE will experience significant changes, and these can be used as important indices for the diagnosis of this disease.

To sum up, CCCI patients often concomitantly suffer from mild depression and cognitive injuries of varying degrees, the CRP, S100B, MBP, HSP-70 and NSE in serum will increase significantly, and the diagnosis of CCCI can be made according to the neuropsychological characteristics and the changes in serum markers.

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Impact of mechanical ventilation on quality of life and functional status after ICU discharge: A cross-sectional study

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Summary

Objective: To evaluate the impact of the need for mechanical ventilation (MV) and its duration throughout ICU stay on the quality of life (QoL) and physical functional status (PFS) after the immediate ICU discharge.
Method: This was a cross-sectional study including all subjects consecutively discharged from the ICU during 1-year period. During the first week after ICU discharge, QoL was assessed through WHOQoL-Bref questionnaire and PFS through the Karnofsky Performance Status and modified-Barthel index, and retrospectively compared with the pre-admission status (variation [Δ] of indexes).
Results: During the study, 160 subjects met the inclusion criteria. Subjects receiving MV presented PFS impairment (Δ Karnofsky Performance Status [-19.7 ± 20.0 vs. -14.9 ± 18.2; p=0.04] and Δ modified-Barthel index [-17.4 ± 12.8 vs. -13.2 ± 12.9; p=0.05]) compared with those who did not receive MV. Duration of MV was a good predictor of PFS (Δ Karnofsky Performance Status [-14.6-1.12 * total days of MV; p=0.01] and Δ modified-Barthel index [-14.2-0.74 * total days of MV; p=0.01]). QoL, assessed by WHOQoL-Bref, showed no difference between groups (14.0 ± 1.8 vs. 14.5 ± 1.9; p=0.14), and the duration of MV did not influence QoL (WHOQoL-Bref scale [14.2-0.05* total days of MV; p=0.43]).
Conclusion: Need for MV and duration of MV decrease patient PFS after ICU discharge.

Keywords: Respiration, Artificial. Quality of Life. Intensive Care Units. Patient Discharge. Activities of Daily Living. Recovery of Function. Cross-Sectional Studies.

Introduction

Critically ill subjects are often exposed to prolonged bed rest, dysfunction of vital organs, sepsis, hypoxemia and neuromuscular drug toxicity, resulting in an impaired cardiovascular system status and critical illness neuromuscular syndromes.[1] Both of these conditions may delay ventilator weaning and increase ICU period and hospital stay.[1] In particular, prolonged immobility and inactivity may result in the loss of muscle strength, endurance, balance and neuromuscular coordination, leading to further total functional impairment and a consequently reduced quality of life (QoL).[1-3] It is suggested that some subjects benefit from critical care therapy, but many others do not.[4]

Mechanical ventilation (MV) is the highest priority indicator for admission to ICUs, according to accepted guidelines.[5] Subjects who require MV are usually the most severe ones. In addition, increasing numbers of these patients generate particular controversy regarding their uncertain long-term outcomes and disability. The greater is the duration of MV, the worse the prognosis appears to be.[6-8] Some authors described that MV subjects experience poor survival, low QoL, reduced physical functional status (PFS) and poor cognitive functioning; the subjects also require substantial post-discharge care. Other authors demonstrated a survival benefit with MV.[7]

A clearer description of the outcomes of MV subjects post-discharge can be observed in clinical decision-mak-

ing, institutional planning, payment reform and design of future interventions targeted to these unique subjects.[6,9] There are no studies establishing the relation of PFS and QoL with MV dependency. Therefore, the objective of our study was to verify the impact of MV (need and duration) on QoL and PFS of subjects after immediate ICU discharge.

Method

Design and participants

The present investigation was a cross-sectional study that included all the subjects admitted to and discharged from the ICU of the Ernesto Dornelles Hospital (a 22-bed clinical-surgical ICU) during a 1-year period (from August 2012 to August 2013). Patients < 18 years of age, those who remained in the ICU for < 72 h, those subjected to elective surgery without clinical or surgical complications and those who refused to sign the informed consent were excluded from our study. Eligible subjects who were readmitted to the ICU during the study period were only included once. The study was approved by the research ethics committee of the Federal University of Health Sciences of Porto Alegre (no. 332.519) and consisted of a preliminary analysis of an ongoing multicenter cohort that is expected to include 1,500 participants.

Intervention

Each eligible patient, or a close relative, was requested to sign the informed consent form during the first week following discharge from the ICU. The subjects who agreed to participate were subjected to an interview with physical therapists and psychologists previously trained to apply the following questionnaires and scales to assess the participants' current condition: PFS (modified-Barthel index and Karnofsky performance status) and QoL (WHOQoL-Bref). The PFS was also assessed in the period prior to ICU admission, retrospectively. The Portuguese translations of all these scales have already been validated.[10-12]

Physical-functional status evaluation

The modified-Barthel index objectively assesses the degree of dependence of individuals relative to 10 categories of activities of daily living (ADLs): personal hygiene, bathing, feeding, toilet use, climbing stairs, dressing, bladder and anal sphincter function, walking, and transfer from bed to chair.[13,14] The score ranges from 0 to 100 and is interpreted as follows: 0-20, totally dependent; 21-60, severely dependent; 61-90, moderately dependent; 91-99, slightly dependent; and 100, totally independent.[13,15,16] The questionnaire can be answered by the subjects, their relatives, or their caregivers. For the present analysis, the absolute values (from 1, totally dependent, to 5, totally independent) of each domain were used.

The Karnofsky Performance Status assesses the degree of functional impairment. It was initially designed to assess the physical performance of subjects with cancer, but its use was extended to other chronic disabling diseases.[14] Based on their scores, the individuals were classified as follows: 100, normal, having no complaints and no evidence of disease; 90, capable of normal activity and with few symptoms of disease; 80, normal activity with some difficulty and some symptoms of disease; 70, capable of self-care and not capable of normal activity or work; 60, occasionally requires some assistance but can take care of most personal needs; 50, requires considerable assistance or frequent medical care; 40, disabled and requires special care and assistance; 30, severely disabled, with indicated hospital admission, although death is not imminent; 20, very ill, requiring hospital admission; and 10, moribund, with fatal process progressing rapidly.[11]

Quality of life evaluation

Data on QoL were collected using the World Health Organization Quality of Life Bref-Scale (WHOQoL-Bref). The WHOQoL-Bref is a shorter version of the original WHOQoL-100 and consists of 26 items that are scored over four major domains, namely physical, psychological, social relationships, and environment.[12,17-19] The responses of the WHOQoL-Bref are scored in a Likert-type scale yielding 1 to 5 points, with higher scores denoting higher QoL and vice-versa.[18,20]

Outcome measures

The information relative to the participants, ICU stay was collected from their clinical records including the following data: demography, severity scores, reason for ICU admission, diseases before ICU admission, requirement for life support (e.g., invasive or non-invasive MV hemodialysis; vasopressors such as dopamine, noradrenaline and dobutamine; or blood-component transfusions such as red-blood-cell concentrates, plasma, and platelets) and ICU outcomes.

Data analyses

The data are expressed as mean ± standard deviation (SD) or absolute and relative frequencies. The Kolmogorov-Smirnov test was used to investigate the normal distribution of the data. The categorical variables were analyzed using Fisher's exact test, and the quantitative

variables were analyzed using Wilcoxon-Mann-Whitney test. The comparison between the differences in score variation [score post-ICU immediate discharge (score before ICU admission [Δ]) in the Karnofsky Performance Status and modified-Barthel index scales and the average WHOQoL-Bref scores between subjects who required MV and those who did not were performed using the Wilcoxon-Mann-Whitney test. Linear regression was conducted to assess the impact of the length of MV on QoL scores. The significance level was established as $p<0.05$. The analysis was performed using Stata software version 12 (Stata Corp LP, USA).

Results

During the study period, 160 subjects discharged from the ICU were included in the analyses. The data corresponding to the participants' ICU stay are described in Table 1, and the following results stood out: subjects with MV were younger (69.5±15.3 years vs. 73.8±14.9 years; p=0.04) and had a higher ICU stay length (8.9±5.4 vs. 5.8±3.0 days, p<0.001).

The results from the modified-Barthel index indicated a tendency of higher difference in score variation of subjects with MV dependence compared with those of subjects without MV (Δ = -17.4±12.8 vs. -13.2±12.9; p=0.05). Furthermore, the Karnofsky Performance Status revealed a poorer functional capacity of subjects with MV after immediate ICU discharge (Δ = -19.7±20.0 vs. -14.9±18.2; p=0.04). However, the WHOQoL-Bref scores showed no difference between groups (14.0±1.8 vs. 14.5±1.9; p=0.14).

Table 2 describes the individual variation of each ADLs category in the modified-Barthel index. The comparison of the categories before and after ICU showed that the group of subjects with MV had a poorer performance in dressing and climbing stairs. Table 2 also describes the WHOQoL-Bref domains, which showed no difference between groups.

The duration of MV was a good predictor of PFS impairment in the immediate ICU discharge (Figures 1A and 1B), but not of QoL (Figure 1C).

Discussion

The main finding of the present study is that MV during ICU stay is an indicator of poor physical functional capacity immediately after ICU discharge.

Impairment has been reported in the ADLs of almost all ICU survivors in studies that evaluated subjects immediately after ICU discharge.[21-23] Nevertheless, the literature is controversial regarding the association between PFS and the MV need. Some authors suggested that functional status during post-hospital follow-up does not seem to be influenced by the use of MV.[24] Other study reported that ICU survivors have a reduced functional capacity in ADLs immediately after ICU discharge, and these limitations are associated with the duration of

TABLE 1 Characteristics of the participants.

Variables	Subjects with MV (n=107)	Subjects without MV (n=53)	p
Male gender, n (%)	64 (59.8)	28 (52.8)	0.49
Age (years), mean ± SD	69.5±15.3	73.8±14.9	0.04
Body mass index (kg/m^2), mean ± SD	26.5±5.9	27.0±7.2	0.71
Previous diseases, n (%)			
Heart failure	17 (15.8)	10 (18.8)	0.65
Ischemic heart disease	6 (5.6)	4 (7.5)	0.73
Diabetes mellitus	20 (18.6)	13 (24.5)	0.41
Peripheral artery disease	5 (4.6)	3 (5.6)	0.71
End-stage chronic kidney failure	5 (4.6)	0 (0)	0.17
Cerebrovascular disease	16 (14.8)	8 (15.0)	0.99
Dementia	10 (9.3)	5 (9.4)	0.99
Bronchial asthma	3 (2.8)	5 (9.4)	0.11
Chronic obstructive pulmonary disease	15 (14.0)	11 (20.7)	0.36
Depression	7 (6.5)	3 (5.6)	0.99
APACHE-II, mean ± SD	20.1±7.9	18.0±6.0	0.08
ICU length of stay (days), mean ± SD	8.9±5.4	5.8±3.0	<0.001

MV: mechanical ventilation; SD: standard deviation; APACHE-II: Acute Physiology and Chronic Health Evaluation II; ICU: intensive care unit.
Note: Difference between qualitative variables evaluated by Fisher's exact test. Difference between quantitative variables evaluated by the Wilcoxon-Mann-Whitney test.

TABLE 2 Comparison+ of the score variation* in categories of modified-Barthel index and comparison+ of the WHOQoL-Bref domains between critically ill subjects receiving or not mechanical ventilation.

Barthel's categories	Subjects with MV (n=107)	Subjects without MV (n=53)	p
Feeding	-1.58±1.38	-1.24±1.43	0.10
Bathing	-1.71±1.30	-1.32±1.42	0.05
Dressing	-1.82±1.63	-1.22±1.58	0.04
Personal hygiene	-2.09±1.66	-1.69±1.61	0.13
Anal sphincter function	-1.91±1.62	-1.47±1.51	0.09
Bladder function	-1.44±1.46	-1.00±1.37	0.06
Toilet use	-1.46±1.76	-1.05±1.59	0.17
Transfer from bed to chair	-1.36±1.65	-1.13±1.72	0.28
Walking	-2.0±1.64	-1.54±1.55	0.08
Climbing stairs	-1.84± 1.47	-1.26±1.44	0.01
WHOQoL-Bref domains	**Subjects with MV (n=60)**	**Subjects without MV (n=34)**	**p**
Physical	11.7±3.1	12.3±2.1	0.48
Psychological	14.8±2.1	14.8±2.5	0.92
Social relationships	15.9±2.6	17.0±1.9	0.07
Environment	14.9±2.2	14.4±2.5	0.29

MV: mechanical ventilation. Variables expressed as mean ± standard deviation.
+ Wilcoxon-Mann-Whitney test.
* Score variation = score post-ICU immediate discharge – score before ICU admission.

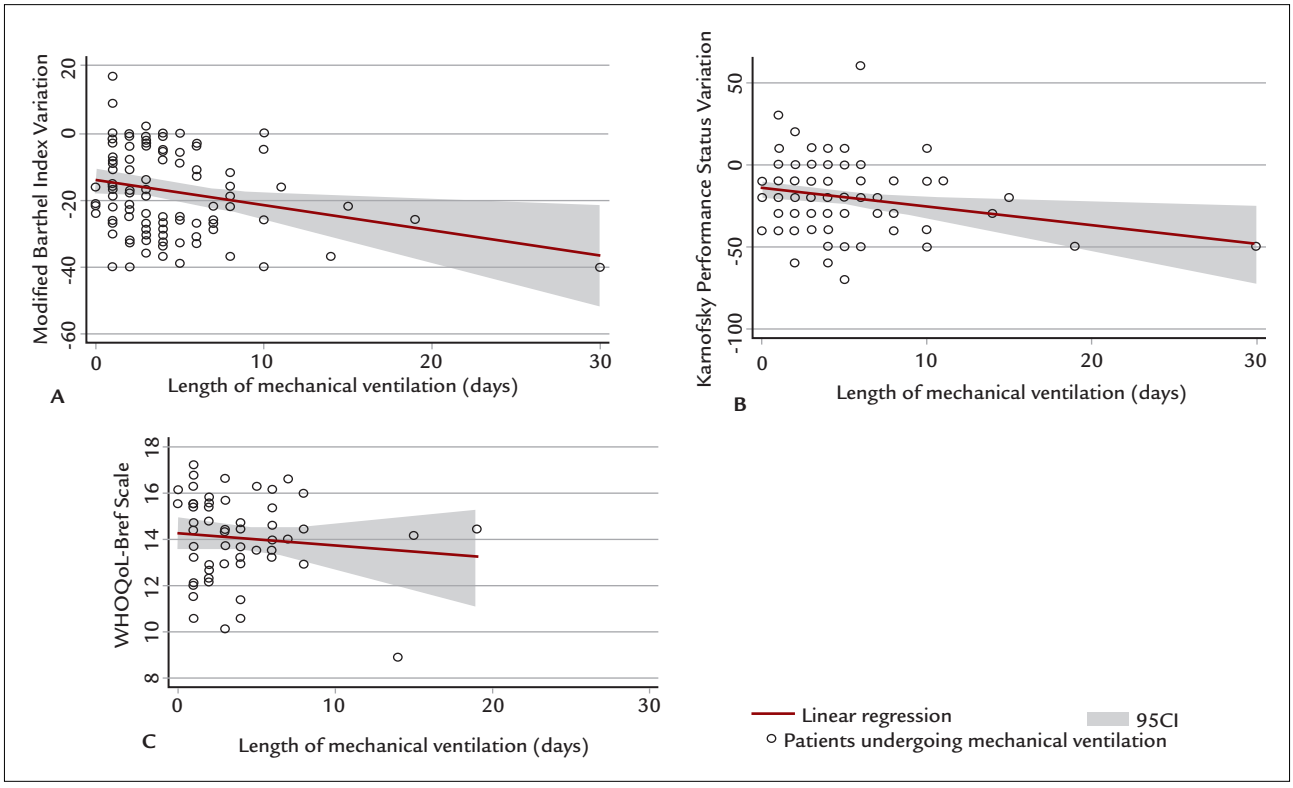

FIGURE 1 A. Linear regression between duration of mechanical ventilation and the score variation of modified-Barthel index+. B. Linear regression between duration of mechanical ventilation and the score variation of Karnofsky Performance Status++. C. Linear regression between duration of mechanical ventilation and WHOQoL-Bref scale+++.

Note: +Score variation = score post-ICU immediate discharge − score before ICU admission. Score variation of modified-Barthel index = −14.2–0.74* total days of MV; p = 0.01. **Score variation = score post-ICU immediate discharge − score before ICU admission. Score variation of Karnofsky performance status = −14.6–1.12* total days of MV; p=0.01. *** WHOQoL-Bref scale = 14.2–0.05* total days of MV; p=0.43.

MV.[25] A previous study by our group demonstrated that the use of MV ≥ 8 days reduced the ability to perform ADLs by 1.48 times (RR = 1.48; 95CI 1.02-2.15; p=0.03).[10] ADLs impairments may be more prevalent in MV subjects because 86% of subjects ventilated for ≥ 48h had limitations in physical function, and approximately 75% of these limitations were severe by the 12th month after discharge.[26] Our study also demonstrated the loss of PFS associated with the use of MV. We believe that because MV is used in more severe cases, which require longer ICU stay, these patients are more often exposed to hypoxemia, bed immobility, use of sedatives and risk of nosocomial infections (not measured by us). The use of MV is not complication-free, affecting the patient as a whole by impairing their physical condition and thus worsening their functional capacity.

Most subjects using MV for longer periods and who survive one year report significant deficits in physical functioning, energy, and sleep. More specifically, measures of functional status were significantly worse in MV subjects, especially for subscales which measured mobility, body care, and movement.[9] Walking ability and upper-extremity grip strength were identified as independent-explanatory consequences of poorer PFS.[25] In another study, the authors demonstrated that more than 25% of subjects report restrictions in activities related to walking such as walking slowly and having problems with walking stairs, hills, and distances.[14] These results corroborate ours, in which subjects of the MV group also showed deficit in climbing stairs and dressing.

Studies assessing QoL after intensive care suggest that this parameter improves over time, but is worse than that before the ICU admission, and worse than expected for the general population.[27-31] The association between QoL and MV remains unclear. Subjects using long-term MV had consistently worse overall QoL than short-term subjects, but the differences were not statistically significant.[9] Another study that compared long-term and short-term MV found that, in the analysis of the physical domain, the long-term MV group showed worse results, specifically in mobility and body care.[32] A 3-year follow-up study showed that subjects that used MV for > 14 days, and another study with subjects who remained in the ICU for > 24h, found no correlation between the duration of MV with overall QoL.[33,34] Some authors described that intubation was not significantly associated with either physical or mental domain scores one month after ICU discharge.[35] In our study, MV showed no association with QoL at immediate ICU discharge. Most of our subjects underwent short-term MV, which may have influenced our results. It is also known that subjects who are in stable conditions after ICU stay may be more likely to express positive perceptions in their QoL than subjects with unstable disease.[19] It is important to note that QoL is a dynamic and variable phenomenon across different subjects and presents time variation for the same patient.[36] In any case, the ideal time to evaluate QoL has not yet been established.[30,35,37,38] Although many of the subjects reported a decline in functional status and many other symptoms, they were satisfied with their QoL. The majority of survivors described their health as good or better and would opt for MV support again if they had to relive the experience.[38] However, it is unknown if family members think the same way.[39]

The immediate post-discharge period remains an important target for improving outcomes and disease management models and should be further evaluated.[9] It is believed that the care provided in ICU settings and the assessment of the interventions performed should be determined earlier along the interval between discharge from the ICU and discharge from the hospital. These measures would have a long-term impact on the QoL of critically ill subjects.[40] Although it is known that no single variable such as the use of MV has a significant and accurate prognosis, it may have implications for patient care, family planning and decision-making.[32] The simple health grouping model outcomes we reported may help in this regard.

Concerning the limitations of our study, we should first mention the selected assessment method (i.e., the use of questionnaires). Although this technique is not subjective, it depends on the individuals' reading and understanding skills, their honesty and their hearing capacity during the interviews; also, the questionnaires may present measurement bias. Moreover, a memory bias should be considered if the questionnaires included information regarding the patient's conditions before ICU admission. In this context, it is noteworthy that survivors of severe diseases might overestimate their state before admission, as reported by other authors.[38,39] The large number of subjects excluded should be addressed, which led to a small sample that may not be representative, especially because few of the study's subjects used MV for more than 10 days. Furthermore, our study was conducted in a single center, as a preliminary analysis of an ongoing multicenter cohort.

Quick look

Mechanical ventilation is an indicator of poor physical functional capacity after ICU discharge.

Mechanical ventilation can decrease the quality of life after ICU discharge.

Duration of mechanical ventilation decreases the physical functional status after ICU discharge.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Impacto da ventilação mecânica na qualidade de vida e no estado funcional após alta da UTI: um estudo transversal

Objetivo: Avaliar o impacto da necessidade de ventilação mecânica (VM) e sua duração na qualidade de vida (QV) e no estado funcional físico (EFF) dos pacientes após a alta imediata da UTI.
Método: Estudo transversal incluindo todos os pacientes que, consecutivamente, tiveram alta da UTI durante um período de um ano. Durante a primeira semana após a alta da UTI, a QV foi avaliada através do questionário WHOQoL-Bref e o EFF através do índice de Karnofsky e do índice de Barthel modificado, comparados retrospectivamente com o estado pré-admissão (variação [Δ] dos índices).
Resultados: Durante o estudo, 160 indivíduos preencheram os critérios de inclusão. Os indivíduos submetidos a VM apresentaram maior prejuízo no EFF (Δ Karnofsky [-19,7 ± 20,0 vs. -14,9 ± 18,2; p=0,04] e Δ Barthel modificado [-17,4 ± 12,8 vs. -13,2 ± 12,9; p=0,05]) quando comparados aos pacientes sem VM. A duração da VM foi um bom preditor de redução do EFF (Δ Karnofsky [-14,6-1,12 * dias totais de VM; p=0,01] e Δ Barthel modificado [-14,2-0,74 * dias totais de VM; p=0,01]). A QV, avaliada pelo WHOQoL-Bref, não mostrou diferença entre os grupos (14,0 ± 1,8 vs. 14,5 ± 1,9; p=0,14) e a duração da VM não influenciou a QV (WHOQoL-Bref [14,2-0,05 * dias totais de VM; p=0,43]).
Conclusão: A necessidade e a duração do VM reduzem a *performance* física dos pacientes após a alta da UTI.

Palavras-chave: Respiração Artificial. Qualidade de Vida. Unidades de Terapia Intensiva. Alta do Paciente. Atividades Cotidianas. Recuperação de Função Fisiológica. Estudos Transversais.

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Neck circumference in adolescents and cardiometabolic risk: A sistematic review

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Summary

Objective: To critically analyze articles on the relation between neck circumference (NC) in adolescents and: body mass index, fat distribution, metabolic syndrome and its individual components, and cardiovascular risk.
Method: Systematic review undertaken by two independent researchers using the Pubmed/Medline, Lilacs/Medline, Scielo and Cochrane databases in English, Spanish and Portuguese in the period comprising the past 5 years.
Results: Eighteen (18) articles were selected. The articles show an association between NC in adolescents and body fat (BMI), central fat distribution (WC), metabolic syndrome and several of its individual components, and cardiovascular risk. Some values are proposed for NC cutoff points as a diagnostic tool for nutritional status, high blood pressure and pre-hypertension, cardiovascular risk, insulin resistance and metabolic syndrome. We identified a percentile curve constructed for Brazilian adolescents.
Conclusion: There is a shortage of studies with representative samples, variety at the NC measurement sites, and the age of the participants, which makes it difficult to establish definitive landmarks.

Keywords: Neck. Adolescents. Metabolic Syndrome X. Obesity. Cardiovascular Diseases. Obesity. Waist Circumference. Anthropometry. Insulin Resistance. Hypertension. Triglycerides. Cholesterol. Blood Glucose. Review.

Introduction

Obesity among adolescents is becoming increasingly prevalent and worrying worldwide due to the increased risk of complications in adulthood or even earlier, during adolescence itself.[1-3] Both the amount and the distribution of body fat are related to insulin resistance (IR), dyslipidemia, high blood glucose and cardiovascular diseases.[4-6]

Direct measurements of body fat (densitometry, electrical bioimpedance, computed tomography, magnetic resonance imaging) are costly and not readily available in clinical practice. Body mass index (BMI) and waist circumference (WC) are the most commonly used measurements in medical appointments because they are uncomplicated and accessible. The former indicates the amount of body fat and the latter, its location, without however distinguishing between visceral and subcutaneous fat types.[7,8]

WC measurement, in turn, may be affected by factors such as an increase in the postprandial period and variation with respiratory movements, or cause embarrassment due to exposure of the abdomen. In addition, this type of measurement does not yet rely on a standardized methodology or reference values defined for adolescents.

Neck circumference (NC) then appears as a more consistent alternative for assessing central fat distribution, originally, in adults[8-11] and, more recently, in adolescents.[12] NC correlates with cardiometabolic risk as much as visceral fat does.[10]

This measurement is socially accepted, has intra- and inter-examiner reproducibility,[13] but should be avoided

in patients with conditions that increase the volume of the cervical region such as in patients with goiter.

Our systematic review aimed at describing and critically analyzing articles on the relation between NC in adolescents and their BMI, fat distribution, metabolic syndrome (MetS) and its individual components, and cardiovascular risk (CVR).

Method

Our review aims at providing access to and a critical analysis of the most current information on the subject.

We designed it in accordance with the Prisma guidelines for systematic reviews: formulation of a problem, bibliographic review, selection of articles, analysis of data and presentation of the review.[14] For this, two independent researchers did a search on January 20th, 2016. We used the following Health Science Descriptors (DeCS, Portuguese acronym for Descritores em Ciências da Saúde) to search the Lilacs/Medline and Scielo databases: neck, adolescent, obesity, waist circumference, anthropometry, cardiovascular diseases, metabolic syndrome, insulin resistance, high blood pressure, triglycerides, cholesterol, blood glucose. The terms we used for the search in the Cochrane database were neck and adolescent. In the case of Pubmed/Medline, we used the following Medical Subject Headings (MeSH): adolescent, obesity, body mass index, adiposity, anthropometry, cardiovascular diseases, metabolic syndrome, hypertension, insulin resistance, triglyceride, high blood glucose.

We selected the articles according to their title and abstract, using the eligibility criteria. The inclusion criteria were: study languages (English, Spanish, and Portuguese); and time filter: 5 years; target population: humans, adolescent as defined by the WHO criteria (10 to 19 years of age); outcome of interest: relation between NC (dependent variable) in adolescents and overweight/obesity, visceral fat/WC, MetS and its components, CVD (independent variables). The exclusion criteria were based on the type of study: editorial, case report, expert opinion.

Both researchers read the selected articles in full and analyzed them. Occasional divergence in selection was resolved by consensus. Upon reading the articles, we carried out a secondary search with the selection of articles from the bibliography contained in the primary documents of the previous search, respecting the inclusion criteria. Figure 1 summarizes the steps of article selection we used for our review.

Results

Characterization of studies

We systematized the main features of the selected articles in one table (Table 1).

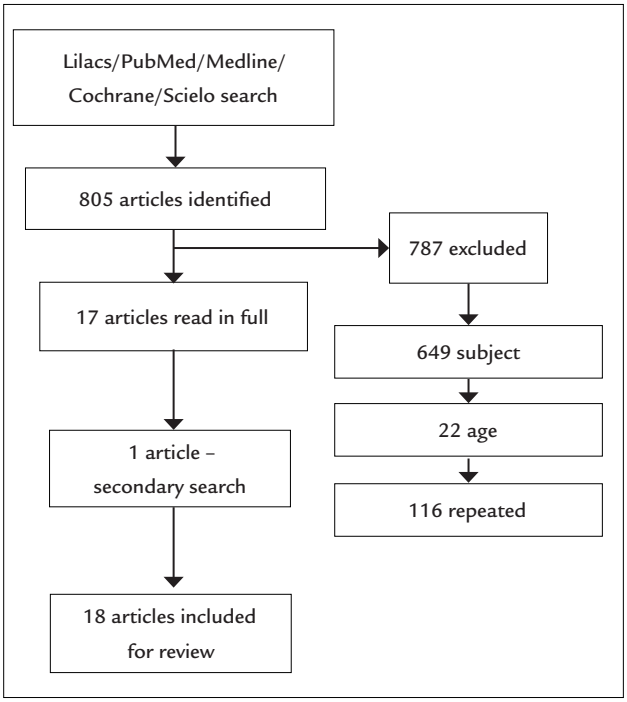

FIGURE 1 Article search flow.

We varied the samples from the selected studies (150 to 6,802, median 1,507 subjects), five of which were random. One of the studies[15] explained that a sample had been chosen out of convenience because of difficulty in adherence.

Most of the studies selected their patients from the same schools where they studied,[15-23] with only four of them involving students coming from public schools,[15,19,20,22] whereas other four used data from patients who were already being followed up in an outpatient clinic.[8,24-26]

Two studies evaluated the same population of inpatients awaiting non-cardiac elective surgery[27,28] and one study obtained a sample from the general population.[29] Brazil was the country of origin of the largest number of publications selected (37.5%), but China had the largest number of subjects studied.

NC and the technical aspects to measuring it

Seventy-two percent (72%) of the studies performed measurements at the level of the thyroid cartilage, an easily identifiable reference landmark. The other measurement sites were: just below the thyroid cartilage,[23] the cricoid cartilage[8,30] and at halfway the height of the neck.[21,26]

In only three studies the measurements had been taken as duplicates or triplicates,[15,23,25] a fact of little relevance due to the already well-documented reproducibility of this type of measurement.[13] Eleven (11) studies[8,16,17,19,20,22,23,27-29,31] mentioned the training of examiners. In one study,[17] repro-

TABLE 1 Characteristics of included trials in the review.

Author, year, country, reference	Sample (n)	% Obese and overweight	Type of sample (random or not)	Age (years)	Measurement at thyroid cartilage	Study design, classification	Level of evidence	Comparison	Suggested cutoff points (cm)
Nafiu et al., 2010, USA[27]	1,102	37.7%	no	6-18	yes	Cross-sectional	4	BMI	For high BMI male from 28.5 to 39.0 female from 27.0 to 34.6
Hatipoglu et al., 2010, Turkey[24]	967	42.7%	N/I	6-18	yes	Cross-sectional	4	BMI, WC, BP	For high BMI male from 28.0 to 38.0 female from 27.0 to 34.5
Mazicioglu et al., 2010, Turkey[16]	4,581	N/I	yes	6-18	yes	Cross-sectional	4	BMI	Percentile curve construction
Lou et al., 2012, China[17]	2,847	36%	yes	7-12	yes	Cross-sectional	4	BMI, WC	For high BMI male from 27.4 to 31.3 fem from 26.3 to 31.4
Phan et al., 2012, USA[8]	152	100%*	no	9-18	no**	Retrospective cohort	3B	BMI, WC, HC, EB****	Mean NC 35.71
Kurtoglu et al., 2012, Turkey[25]	581	79.3%	no	5-18	yes	Cross-sectional	4	MetS according to IDF, BMI, WC, HOMA-IR	For MetS male at 36 female at 35
Hingorjo et al., 2012, Pakistan[18]	150	26.6%	no	18-20	yes	Cross-sectional	4	BMI, WC, HC	For high BMI male 35.5 female 32
Guo et al., 2012, China[19]	6802	23.1%	Yes	5-18	Yes	Cross-sectional	4	Pre-HBP, BMI, WC	For pre-HBP 33.2 (p90)
Silva et al., 2014, Brazil[26]	260	50.8%	No	10-19	No***	Cross-sectional	4	IR (HOMA IR), BMI, WC, EB***, components of MetS	For IR male prepubescent 30.3 and pubescent 34.8 fem prepubescent 32 and pubescent 34.1
Oliveira et al., 2014, Brazil[20]	260	N/I	No	10-19	N/I	Cross-sectional	4	BP	Direct correlation with SBP and DBP in both genders
Gonçalves et al., 2014, Brazil[21]	260	20.4%	Yes	10-15	No***	Cross-sectional	4	WC, BP, DEXA, glycemia, insulin, lipids	For cardiovascular risk male 30.4 female 28.8

(*continues*)

TABLE 1 (Cont.) Characteristics of included trials in the review.

Author, year, country, reference	Sample (n)	% Obese and overweight	Type of sample (random or not)	Age (years)	Measurement at thyroid cartilage	Study design, classification	Level of evidence	Comparison	Suggested cutoff points (cm)
Katz et al., 2014, Canada[29]	1,913	26.3%	Yes	6-17	Yes	Cross-sectional	4	BMI and WC	For high BMI male at 33.3 female at 31.6 Percentile curve construction
Fuly et al., 2014, Brazil[15]	794	23.6%	No	6-13	Yes	Cross-sectional	4	HBP	Mean NC hipertensive 28.9±3.0 normotensive 27.5±2.1
Kuciene et al., 2015, Lithuania[31]	794	15.8%	N/I***	12-15	Yes	Case-control	3D	HBP	BMI and WC For pre-HBP and HBP male 12 years: ≥ 35 / 13 years: ≥ 36 /14 -15 years: ≥ 38 female 12 years: ≥ 33/13 -14 years: ≥ 34 15 years: ≥ 35
Ferretti et al., 2015, Brazil[22]	1,668	29.5%	No	10-17	Yes	Cross-sectional	4	BMI, BP	For overweight / obesity: male 34.25 / 37.95 female 31.25 / 32.65
Androutsus, 2012, Greece[23]	324	37%	No	9-13	No*	Cross-sectional	4	BMI, WC, HC, WC/HC, lipids, BP, IR levels and blood glucose	Mean NC male 30.7±2.54 female 30.5±2.25
Coutinho, 2014, Brazil[30]	2,794	35%	No	6-19	No**	Cross-sectional	4	BMI, WC, WC/HC, EB	Percentage curves: mean±2SD
Nafiu, 2013, USA[28]	1,058	37.7%	No	6-18	Yes	Cross-sectional	4	BMI, CI, WC/H, BP	P90, nutritional status: normal weight 33.6±3.2/overweight 34.9±3.7/obese 36.7±5.2

* 100% obese, ** At cricoid cartilage, *** mean neck length, **** electrical bioimpedance, N/I: not identified, N/A: does not apply.
BMI: body mass index; NC: neck circumference; MetS: metabolic syndrome; BP: blood pressure; HBP: high blood pressure; WC: waist circumference; HC: hip circumference; IR: insulin resistance; CI: conicity index.

ducibility tests of its anthropometric measurements were run. Only six studies[17,18,25-27,30] declared the exclusion of subjects bearer of cervical lesions that could falsify the measurements, such as masses or deformities. In the other articles, exclusion criteria were based on the use of medications[26] or pathologies[19,25,26,30] that could influence cardiometabolic parameters.

NC: age, gender, pubertal stage and nutritional status

Only two studies presented age groups in perfect agreement with our review objectives.[20,26] The remaining studies also included children[8,15-17,19,23-25,27-30] or young adults[18] (Figure 2).

The studies showed homogeneous distribution of the participants between the genders. NCs tended to be larger in males and to increase with age in all studies suggesting cutoff points by gender and/or age. The difference in measurements increased from 11[16,29,30] up to 18 years of age, at which age it was found to stabilize. By then, NCs were 4 cm larger in males than in females.[16]

Pubertal staging was performed in five studies,[22-26] two of which were self-assessed.[22,26] Three studies used these data to identify cutoff points along an ROC curve,[22,24,26] one calculated the mean according to the pubertal stage[22] and yet another study adjusted the data by the degree of pubertal development.[24]

Considering the participants' nutritional status is an extremely important aspect when evaluating NC results. Obese or overweight participants accounted for 20-40% of the sample in 69% of the studies reporting such data (Figure 3).

NC as predictor of cardiometabolic risk factors

The most investigated relation of NC was that with BMI, and it was statistically significant in all articles that evaluated it.[16-19,21,22,24-31] Although this finding was consensual, the way it was assessed was not. As previously mentioned, the NC measurement site varied, as did the landmark used for BMI assessment (CDC,[26-29] WHO[18,21-23,30], Group of Chinese Obesity Task Force 2004,[17] International Obesity Task Force,[16,19,31] unspecified[8,15,24,25]).

The second most investigated relation, whose finding was universal, was that between WC and NC.[17-21,24-31] The most frequent measurement site was the midpoint between the last rib and the iliac crest.[15,17,18,20,21,25-27,30,31] There were also measurements taken at the top of the iliac crest[24,29] at the navel level,[19] whereas another two studies did not explicitly state the measurement site[28] of choice.

Other manners of evaluating body fat were used. A positive association was found between NC and cutaneous folds,[22] electrical bioimpedance,[8,26,30] densitometry,[21] body adiposity index (BAI) (the ratio of WC [waist circumference] [cm] to height [m] multiplied by the square root of the height).[31]

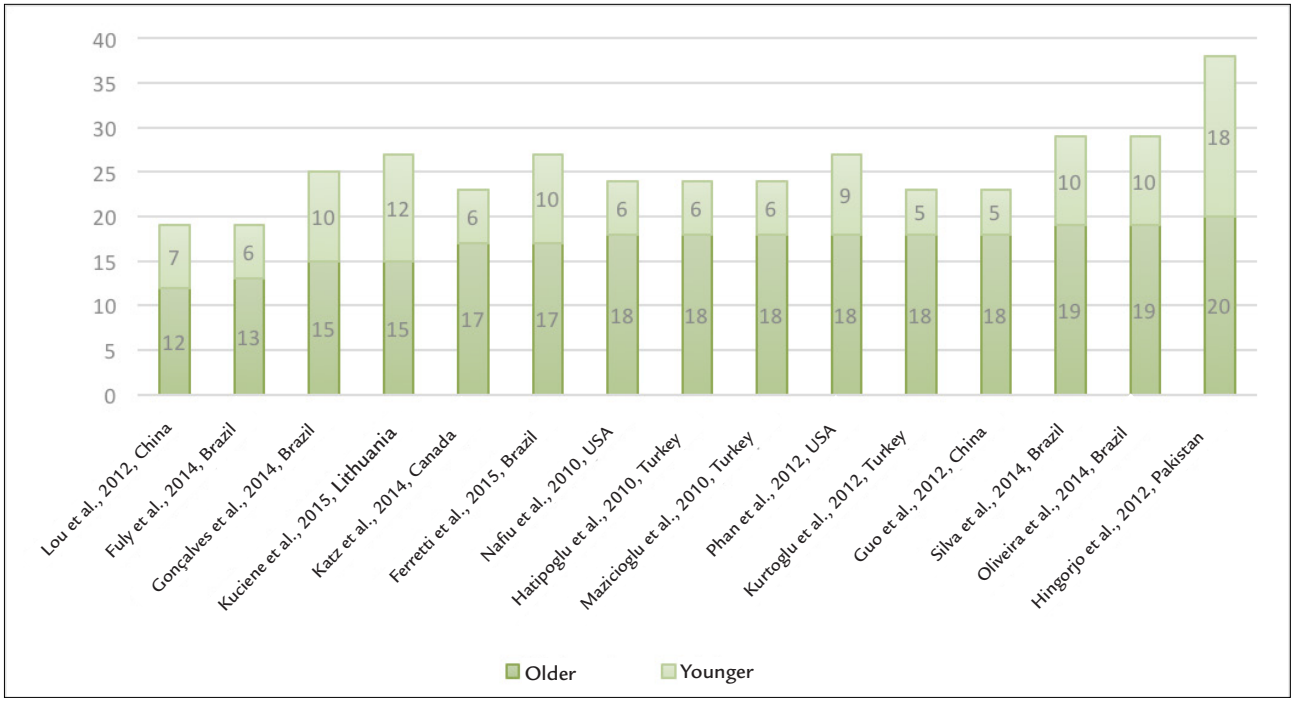

FIGURE 2 Age groups by study.

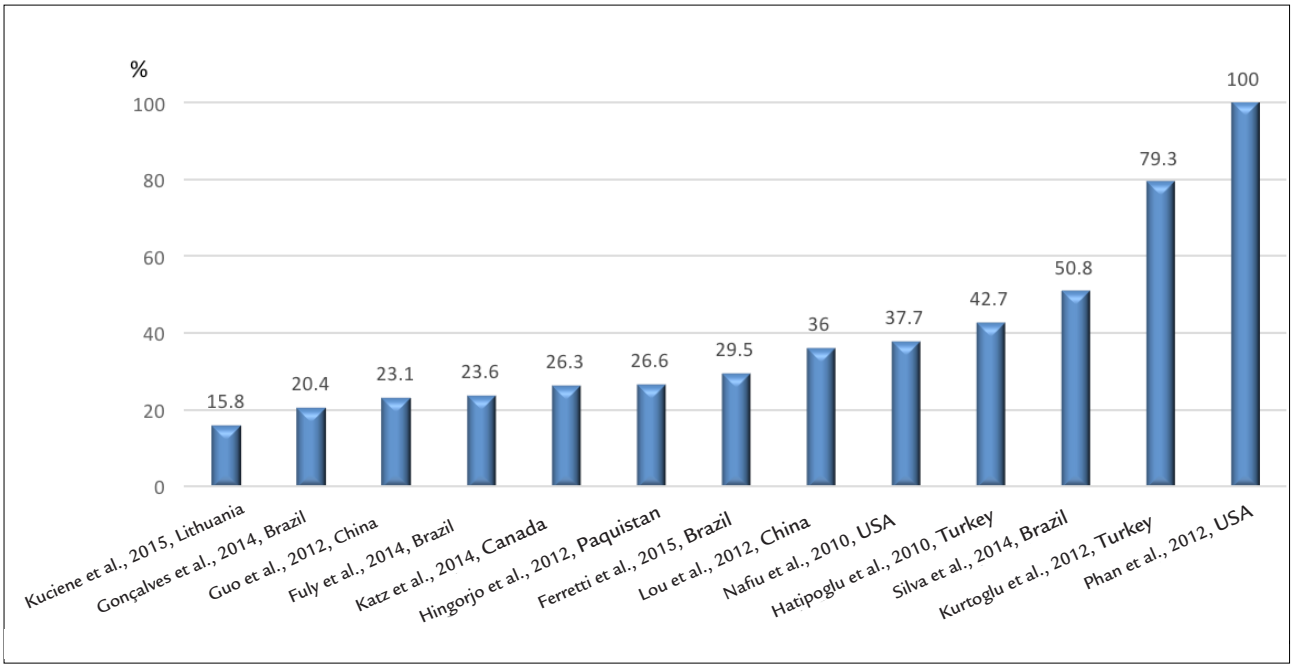

FIGURE 3 Percentage of obese and overweight.

Some studies assessed the relation between NC and IR or several parameters used to diagnose MetS. There was a correlation between NC and IR (HOMA-IR),[21,25,26] fasting insulinemia,[21,25,26] but not blood glucose.[25,26]

With regard to cardiometabolic parameters, a correlation was found with systolic blood pressure (SBP), diastolic blood pressure (DBP)[21,23,25,26] and triglycerides;[21,23,25,26,28] and a negative correlation was found with HDL cholesterol.[21,23,25,26] The correlation with total cholesterol was less consistent, being either positive[8] or negative[21] depending on the study.

NC in Brazil

Our review includes 6,036 Brazilian participants, distributed across six cross-sectional studies. Only one used random sampling[21] and also suggested cutoff points for NC.

The suggested cutoff points are specific for each metabolic parameter evaluated (body fat, triglycerides, HDL cholesterol, blood pressure, fasting blood glucose, fasting insulinemia).

One of the studies[30] suggested NC percentile curves after assessing 2,794 adolescents. Normality was defined as the values included within ± 2 standard deviations of the mean, by gender and age. However, sampling was not random, overweight was more frequent in its population than the national average. Furthermore, pubertal staging was not performed and NC measurements were taken at the cricoid cartilage level. The authors' justification was that the increase in thyroid cartilage size is not unanimous in pubescent boys.

Two studies[22,26] generated specific cutoff points for pubertal staging. In one of them,[26] in which most participants were overweight, the measurement was performed at halfway the height of the neck, with the goal of predicting IR. In yet another group,[22] in which about one-third of the participants were overweight, the measurement was made at the level of the laryngeal prominence and the objective was to predict nutritional status. The findings were close to those obtained by a similar Turkish study.[24]

Discussion

In 1956, when evaluating the neck fold, Vague et al. were pioneers in suggesting that fat distribution in the upper body had clinical implications.[32] Experimental studies corroborated this suggestion by demonstrating increased lipolysis in the presence of fat preferentially stored in the upper body.[5,33] Lipolysis progresses with a release of free fatty acids in excess, insulin resistance in the muscles, an increase in the endogenous production of glucose and VLDL cholesterol by the liver.[34] Clinically, these changes manifest as the components of metabolic syndrome. This state is evident in the presence of excess visceral and adipose tissue, as is the case with neck circumference measurements.[5,6,35,36]

Determining NC reference values for screening overweight and CVR factors in adolescents has some particularities. In addition to the effects of growth per se, pubertal development causes changes in body fat distribution and is associated with a projection of the thyroid cartilage at its midline in males. Thus, setting specific cutoff points by gender and age may be insufficient.

Only one Brazilian study made NC measurement at the thyroid cartilage level (the most frequent site in the selected international literature): it generated cutoff points by gender, nutritional status and pubertal stage[22] (data not shown). This stratification appears to be more important among eutrophic adolescents, whose cardiometabolic risk would not be identified in a BMI assessment.

In both adults and adolescents, the articles selected for our review were unanimous in attesting the correlation between NC and the two measurements most frequently used in clinical practice to estimate adiposity, namely BMI and WC. The correlation still held true, despite the lack of unanimity regarding the anatomical parameters for measuring NC, WC and the reference values for classifying BMI, WC and NC. However, the studies in adolescents are more heterogeneous.

Like the studies in adults, they do consider the difference in NC values between genders, which grows more relevant from puberty on. In addition, since they also usually include the pediatric age group, they present a panorama of growth from the point of view of NC.

As expected, as the age group approaches 18 years, the studies in adolescents suggest that the values for monitoring overweight status get closer to those proposed for adults: 33 to 35 cm for females and 37 to 38.5 cm for males.[9,37-40] This can be better appreciated when comparing the NC percentile scores in Turks between 6 and 18 years of age[16] and in a representative sample of the adult population from the same country.[40]

Cardiovascular diseases are the leading cause of death in adults. Studies in adults have explored NC's ability to predict the risk thereof either directly or by means of their risk factors.[10,37-44]

As seen in adolescents, NC values could be correlated with SBP and DBP,[10,37,39,42,45] triglycerides,[39,41,42,45] IR,[10,37] but also blood glucose,[10,37,39,41,42,45] total cholesterol,[39,41,45] HDL[10,37,39,42] and LDL.[39,41,45]

The proposed values are identical to those indicated for overweight screening: 33 to 35 cm for females and 37 to 39 cm for males.[37-39]

Even after adjustment for adiposity as measured by BMI or WC, NC remained a good predictor for diabetes,[42] insulin resistance[10] and metabolic syndrome,[40] but not for coronary atherosclerotic load.[46]

Obesity in childhood increases morbidity and mortality in adulthood, a fact corroborated by a study with 23.9 years of follow-up.[47] As a matter of fact, there has already been a downward trend in the increase in life expectancy over the last 30 years.[48] Additionally, even before adulthood, obesity can lead to cardiometabolic complications,[49] and NC could serve as an instrument for screening.

Our review presents a compilation of the most current articles on the association of NC to nutritional status and cardiometabolic risk factors in adolescents. While searching through the articles, we located a systematic review from 2014 that included the subject of NC in children.[12] We had already selected all three articles cited in this systematic review in our bibliographic review.

The topic is current and relevant and encourages the introduction of NC measurements in both the clinical practice and epidemiological studies. Nevertheless, there are still some gaps to be filled by new studies. To date, there is still no measurement site defined for taking NC measurements, which thus hampers comparisons between studies. Most studies disregard the effects of pubertal development on NC. Furthermore, there are few studies with random samples. There may also be variations according to ethnicity, similar to WC, which also call for a larger number of studies. On the other hand, our review already shows several proposed landmarks for the screening of overweight/obesity status and cardiovascular risk factors, separated by gender and age group. We also selected articles suggesting percentile curves. In summary, our review, in agreement with articles already published on adults, lists studies demonstrating the association between NC and body fat (BMI), central fat distribution (WC), metabolic syndrome and several of its individual components, and CVR in the adolescent population.

The simple and rapid measurement may be useful for a secondary prophylaxis, serving as the basis for the continuation of propedeutics in adolescents. This is an extremely desirable measure, given its simplicity, low cost and reproducibility, which should inspire further studies that can propose more definitive cutoff points.

Conclusion

The studies we found and our systematic review demonstrate the recent interest in NC in adolescents in several countries for assessing body fat and CVR factors. Inexpensive, simple and reproducible, not only is it able to predict general and localized body adiposity, but also the complications thereof. However, there is a shortage of studies with representative samples, which makes it difficult to establish cutoff values by gender and age group. Routine clinical use

still depends on the standardization of NC measurement and its interpretation. Furthermore, almost all of the studies included in our investigation used a cross-sectional methodology, which limits the determination of cause and effect due to their undefined temporality.

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Individual contributions

Aisha Aguiar Morais and Urjel Bouissou Aguiar Morais carried out the investigation, selection and critical reading of the articles and wrote the integrative review. Joel Alves Lamounier and Márcia Christina Caetano Romano critically reviewed the intellectual content of the manuscript and approved its final version.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Circunferência do pescoço de adolescentes e risco cardiometabólico: uma revisão sistemática

Objetivo: Analisar criticamente artigos referentes à relação entre a circunferência do pescoço (CP) de adolescentes e: índice de massa corporal, distribuição de gordura, síndrome metabólica e seus componentes individuais e risco cardiovascular.
Método: Revisão sistemática realizada por dois pesquisadores independentes nas bases de dados Pubmed/Medline, Lilacs/Medline, Scielo e Cochrane nos idiomas inglês, espanhol e português nos últimos 5 anos.
Resultados: Foram selecionados 18 artigos. Os artigos demonstram a associação entre CP de adolescentes e gordura corporal (IMC), distribuição central gordura (CC), síndrome metabólica e vários de seus componentes individuais, e risco cardiovascular. Existem propostas de pontos de corte da CP para o diagnóstico do estado nutricional, hipertensão e pré-hipertensão, risco cardiovascular, resistência insulínica e síndrome metabólica. Foi identificada ainda uma curva de percentis construída para adolescentes brasileiros.
Conclusão: Há escassez de estudos com amostras representativas, variedade nos locais de medição da CP e na idade dos participantes, o que dificulta estabelecimento de referências definitivas.

Palavras-chave: Pescoço. Adolescente. Síndrome X Metabólica. Doenças Cardiovasculares. Obesidade. Circunferência da Cintura. Antropometria. Resistência à Insulina. Hipertensão. Triglicérides. Colesterol. Glicemia. Revisão.

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Treatment of chikungunya chronic arthritis: A systematic review

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Summary

Introduction: Chikungunya (CHIK) is a tropical arbovirus, transmitted by the female mosquito *Aedes aegypti* and *Aedes albopictus*. In Brazil, there have been cases reported since 2014. The initial manifestations of this virus are sudden onset high fever, headache, chills, rashes, myalgia and intense joint pain. Usually, CHIK presents the acute and chronic phases, the latter characterized by bilateral polyarthralgia, which can last for months or even years. During this period, autoimmune diseases can be triggered, making the picture even more complicated.
Method: A systematic review was performed on the PubMed and Scielo databases in January 2017. Clinical trials, cohorts, case-control and case reports were included in the study. Expert opinions, societal consensuses and literary reviews were exclusion criteria. Studies were conducted in English, Spanish and Portuguese. The studies were descriptively analyzed and the data was grouped according to methodological similarity.
Results: Twenty-four (24) articles were selected and, in compliance with the inclusion and exclusion criteria, 18 were eliminated, with six studies remaining in the present review: five clinical trials and one case report.
Conclusion: When the manifestations of CHIK become chronic and, the longer they last, more complications arise. Polyarthralgia can be immaterial, distancing individuals from their daily-life activities. Anti-inflammatory drugs (either steroid or not), in addition to immunosuppressants, homeopathy and physiotherapy are measures of treatment that, according to the literature, have been successful in relieving or extinguishing symptoms. However, it is fundamental that studies of CHIK treatment be further developed.

Keywords: Chikungunya Virus. Arthralgia. Arthritis.

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Introduction

Chikungunya (CHIK) is a disease caused by the Chikungunya virus (CHIKV), an alphavirus belonging to the family Togaviridae, which is transmitted through the bite of infected *Aedes aegypti* and *Aedes albopictus* female mosquito.[1,2] CHIK is considered a tropical disease because it circulates within the subtropical and tropical regions, with geographic distribution in the African continent, Southeast Asia and South America, with cases in Europe and North America occurring mainly in travelers.[2,3] In 2013, the first case of local transmission in the Americas was reported in the Caribbean. In 2014, Brazil, together with other South American countries, such as Colombia, Suriname and Paraguay, had already registered the local circulation of the virus.[4] Symptomatic infection caused by this virus is usually marked by a sudden onset of fever, with a body temperature normally higher than 39°C, headache, chills, conjunctivitis, rash, myalgia and severe joint pain, with or without swelling.[1-5] Although CHIK may present with different clinical manifestations in the regions where the disease virus circulates, the presence of the debilitating polyarthralgia symptom causes the predictive value for CHIK to be greater than 80%.[1,5]

In general, CHIK has two phases: acute and chronic. During the acute phase, the main manifestations are high fever, headache, chills, nausea, vomiting, fatigue, back

pain, myalgia and arthralgia.[1,2] As for joint symptoms, there is variation from polyarthralgia to symmetrical polyarthritis; the most affected joints are wrist, metacarpal and interphalangeal joints, elbows, ankles, and knees.[2,5,6]

Arthralgia as standard does not present a defined course, but tends to be more intense in the morning, worsening with more intense physical activity.[1] In the chronic phase, the manifestations are similar to those of the acute phase, including the symmetrical involvement of the joints. Joint pain that persists beyond recovery time is what characterizes this phase. It usually stays for two weeks but there is a possibility that polyarthralgia can last from weeks to years.[1,3] Therefore, in the chronic phase, CHIKV can produce severe arthralgia and/or arthritis, lasting months to years after the initial infection, interfering with the individual's quality of life.[3,7]

Chronic arthralgia does not currently have an effective causal relationship with the initial CHIK virus infection, although there is an association between them. Possible causes of chronicity of arthralgia are: viral persistence, genetic predisposition, autoimmune disease induction, tissue damage directly caused by the virus and exacerbation of preexisting joint disease.[3,8] In addition, disease severity in the acute phase is also related to the risk of developing chronic arthralgia in the chronic phase. Some parameters analyzed, which correlate with the chronicity of the symptoms, are the severity of joint pain and the presence of swelling in the joints.[3]

Thus, arthralgia that persists in the chronic phase of the disease is often debilitating and can lead to impairment in the individual's life, with cases in which patients continue with incapacitating arthritis, even affecting their mobility and requiring long-term treatment.[5,9]

Currently, there is no specific treatment for CHIK, although some drugs are used for this purpose, as well as therapeutic techniques. The objective of our study was to conduct a systematic review of the literature regarding the main types of current treatments for arthralgia in the chronic phase of chikungunya.

Method

The study was based on the Cochrane manual for the preparation of systematic reviews.[10] The search for studies was done in the electronic databases PubMed and Scielo in January, 2017. To this end, the keywords "chikungunya and treatment and arthralgia" and "chikungunya and treatment and arthritis", available in the MeSH – Medical Subject Headings, were entered in both data platforms, without language restriction for articles. The research protocol used as inclusion criteria: clinical trial articles, cohort study, case-control, experimental, descriptive, case series and single case report, all published in the last ten years, excluding: literature reviews, consensuses of medical societies, expert opinion and articles of restricted access.

The articles identified by the initial search strategy had their titles and abstracts evaluated independently by two researchers, authors of this study, obeying the inclusion and exclusion criteria referring to the research protocol; there was no restriction to population groups, and only studies with humans that presented a relevant outcome for the subject were included. In cases where reading the abstracts was not enough to determine their eligibility according to defined inclusion criteria, the articles were read in full so that they could be included or excluded.

In order to extract information from the selected studies, an instrument was prepared, including the following information: authors, year of publication, methodological design, intervention protocol, intervention groups, sample, time and frequency, and outcome. From that point on, the studies were analyzed and interpreted descriptively and their data were presented based on methodological similarities, grouped according to the final conclusions.

Results

To obtain the results, after the research based on the keywords established in the research protocol, 20 articles were selected in the PubMed platform and 4 in the Scielo, which referred to the proposed theme, totaling 24 articles. Of these, two were eliminated because they were duplicated, five because they did not constitute human studies, seven because they corresponded to literature reviews, two because they represented opinion articles, and two because they were not accessible for reading. Thus, six studies were included in our systematic review (Figure 1).

The studies selected for review of the literature were published between 2008 and 2016, with three studies conducted in India,[9,11] Asia; one in Reunion Island,[12] East Africa; one in the Dominican Republic,[13] Central America; and one in Brazil,[14] South America.

As for characteristics of methodological design, five studies were clinical trials, three of them contemplating the population group between 12 and 80 years,[11,12,15] while two did not describe the age of the sample group.[9,14] In the case of clinical trials, treatment outcome results with the following types of drugs were assessed: steroidal and non-steroidal anti-inflammatory drugs (SAIDs and NSAIDs), disease-modifying antirheumatic drugs (DMARDs) such

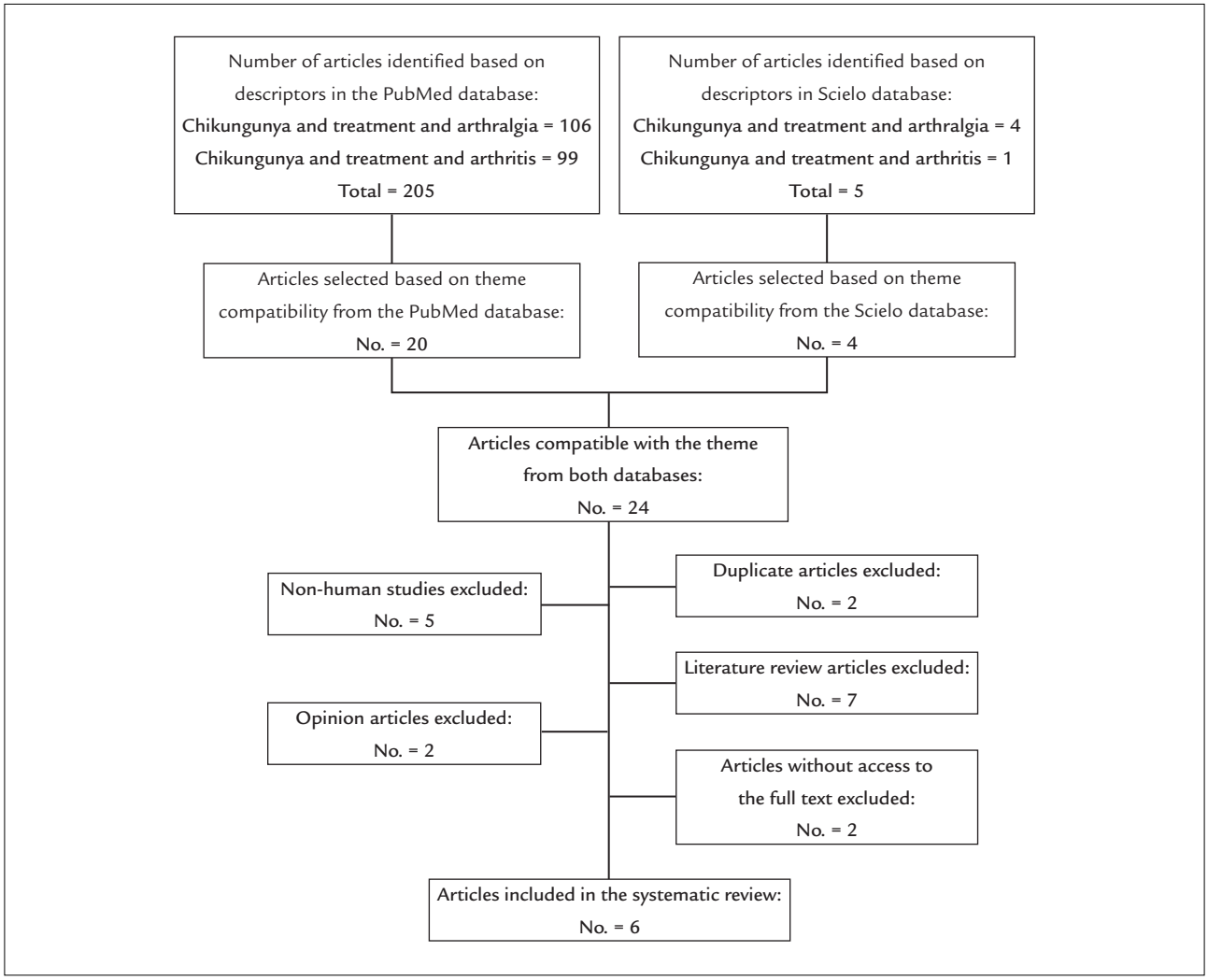

FIGURE 1 Flowchart for identification and selection of articles of systematic review on the treatment of arthralgia in the chronic phase of chikungunya.

as hydroxychloroquine, chloroquine, ribavirin, immuno--modulators and homoeopathic substance (Table 1).

Another study, structured as a case report, presented a non-pharmacological strategy using ultrasound, TENS, and laser using a physiotherapeutic approach (Table 1). This study revealed the potential of such techniques in reducing the pain experienced by the patient reported, reflected in the decrease in the use of oral analgesics.[14]

Among the pharmacological approach studies analyzed (Table 2), two presented an evaluation for NSAIDs. In both cases, there was improvement in the symptoms of arthritis with arthralgia and edema after using this class of drug. The study conducted in Reunion Island reported an improvement in symptoms in 70% of patients after the use of NSAIDs; however, the doses, frequencies and period of administration were not reported, not even the drug.[12]

The study in the Dominican Republic, in turn, showed improvement of symptoms in 89% of patients who used NSAIDs, including: Naproxen with 550 mg twice daily; celecoxib with 400 mg daily, and etoricoxib with 90 mg daily.[13]

The group studied in Reunion Island was also evaluated for the use of methotrexate, with improvement in symptoms in 75% of cases, and hydroxychloroquine, which did not present benefits.[12] The study performed in the Dominican Republic showed a 72% improvement in the use of SAIDs such as prednisone at a dose of 5-7.5 mg daily and flazacort at a dose of 6 mg daily.[13]

Chopra et al.[11] conducted a double-blind randomized trial of patients who presented arthritis more than six weeks after CHIKV infection to assess the benefits of chloroquine over NSAID meloxicam. In the study, the authors found no statistical difference between the two drugs.[11]

TABLE 1 Stratification of studies, available from online databases, in January 2017.

Author, year	Country	Design	Sample	Drugs used
Javelle et al.[12]	Reunion Island	Clinical trial	159, aged 16 to 80 years	SAIDs, methotrexate and hydroxychloroquine
Chopra et al.[11]	India	Clinical trial	70 adults	Chloroquine and meloxicam
Wadhwani[15]	India	Clinical trial	126, aged 12 to 60 years	Homeopathy
Ravichandran and Manian[9]	India	Clinical trial	20	Ribavirin
Rosario et al.[13]	Dominican Republic	Clinical trial	567	NSAIDs and DMARDs
Ribeiro et al.[14]	Brazil	Case report	1	Physiotherapeutic approach

SAIDs: steroidal anti-inflammatory drugs; NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifying antirheumatic drugs.

TABLE 2 Detail of the studies with pharmacological approach January, 2017.

Author, year	Study objective	Detailed methodology	Detailed intervention	Outcome
Javelle et al.[12]	To analyze and evaluate the clinical and therapeutic spectrum effective for rheumatic disorders after CHIKV	A retrospective clinical study that evaluated medical records of patients with rheumatic or skeletal muscle pain after CHIKV infection treated at a rheumatology clinic over 6 years with the identification of clinical and laboratory data before and after the treatment	SAIDs: unspecified dose Methotrexate: 15 mg/week Hydroxychloroquine: 200 mg/day	Patients treated with SAIDs presented a positive therapeutic response in 70% of the cases; treated with methotrexate, 75%. Hydroxychloroquine did not present benefits
Chopra et al.[11]	To evaluate whether chloroquine has greater benefits in relation to meloxicam for the treatment of musculoskeletal pain and post-CHIKV arthritis	A prospective, randomized, double-blind clinical trial evaluating patients with arthritis for more than 6 weeks after CHIKV infection	Chloroquine: 250 mg/day Meloxicam: 7.5 mg/day	There was no difference in efficacy between the groups using chloroquine and meloxicam
Wadhwani[15]	To observe the effect of homeopathic therapy in the acute phase and post-CHIKV chronic arthritis	A prospective clinical trial that evaluated patients in the acute phase of chikungunya and with chronic arthritis after CHIKV	The homeopathic medication was used at initial doses three times a day, being reduced to two and one, with improvement	90% of cases of chronic arthritis achieved cure after an average time of 32.5 days
Ravichandran and Manian[9]	To evaluate the effect of the antiviral drug ribavirin on the clinical outcome of patients with post-CHIKV arthritis	Prospective clinical trial evaluating patients with post-CHIKV arthritis. One group of patients used the drug for 7 days, and was reevaluated after 4 weeks, while the control group used analgesics freely	Ribavirin group: 200 mg twice daily for 7 days. Control group: analgesics, if necessary	At the end of 4 weeks, the ribavirin group showed improvement in walking capacity and edema in 70 and 80% of the cases, respectively, and relapse of pain in 30%, while the control group presented improvement in walking capacity and edema in 30 and 60%, respectively, and 70% of recurrence of pain
Rosario et al.[13]	To compare the therapeutic difference between patients from the general population and those previously diagnosed with rheumatoid arthritis, after CHIKV infection events	A retrospective clinical study evaluated the therapeutic use in a group of patients without rheumatic complaints prior to CHIKV infection and patients undergoing treatment for rheumatoid arthritis after CHIKV	Naproxen: 550 mg twice daily; celecoxib: 400 mg/day; etoricoxib: 90 mg/day; prednisone: 5-7.5 mg/day; deflazacort: 6 mg/day	The population group that had no previous rheumatic complaints had 89% improvement with NSAIDs, 72% with SAIDs

SAIDs: steroidal anti-inflammatory drugs; NSAIDs: non-steroidal anti-inflammatory drugs; CHIKV: Chikungunya virus.

Similarly, the benefits of ribavirin in relation to the control group were evaluated.[13] Patients were treated with ribavirin 200 mg twice daily for seven days, and were evaluated four weeks after therapy, while the control group used analgesics freely during the period. One of the main findings of the study was that after four weeks, the ribavirin group showed improvement in walking ability in 70% of patients, reduction of edema in 80% and recurrence of pain in 30%, while the control group showed improvement in walking capacity in 30%, edema in 60% and recurrence of pain in 70% of patients.[13]

There was also a prospective clinical study using homeopathic approach, in which Wadhwani[15] evaluated the use of homeopathic medication in the acute phase of chikungunya and chronic arthritis caused by the infection. One of the results that could be observed was that, in addition to the benefit in the acute phase, the therapy produced cure of the symptoms of arthritis in 90% of the evaluated cases, with a mean recovery time of 32.5 days.[15]

Discussion

CHIK is a disease that can present with two phases, acute and late. The acute phase is the period in which symptomatic patients generally report abrupt onset, often characterized by high fever, polyarthralgia, back pain, headache and fatigue.[14] The late phase usually manifests with arthralgia or musculoskeletal pain, with more frequent and lasting signs, interfering for weeks or months, and sometimes for years in the patients' quality of life.[1] Despite the various therapeutic regimens available for CHIK, 40% of patients progress with chronic pain and compromised quality of life,[14] making it critical to research on late-phase therapy.

The management of patients with chronic inflammatory rheumatic disease after CHIKV has been reported and appears to bring benefits with the use of methotrexate (MTX). Ribeiro et al.[14] report that MTX may be used at an average dose of 15 mg per week, given the similarities between arthralgia associated with chronic CHIKV and rheumatoid arthritis (RA). In cases of RA after CHIKV, clinical features such as joint destruction and positivity of rheumatoid factor have been described, but in a limited number of CHIK post-fever cases.[2]

Castro et al.[1] argue that the use of MTX is justified by the observation of the presence of monocytes and macrophages in the synovial tissue of chronic patients, perhaps due to the persistence of the virus in this site. Goupil and Mores[3] mention the benefit of treatment of up to six months with MTX and hydroxychloroquine, so that in some cases, magnetic resonance imaging (MRI) showed an improvement in the severity of joint edema, pain and tendon involvement for 15 months after the beginning of treatment.

Another drug that is gaining ground in the treatment of the chronic phase of CHIKV is ribavirin, which is a synthetic nucleoside analog that inhibits a wide range of RNA and DNA viruses. The mechanism of action of this drug is not yet fully understood and may be different for different groups of viruses. In studies by Ravichandran and Manian,[9] ribavirin was used in a group of patients with chronic, incapacitating arthralgia due to CHIKV. The analgesic was discontinued and ribavirin started at 200 mg twice daily for seven days. All patients reported improvement of pain; however, this study presented as a limitation a small number of people, as well as not being a planned study, since the patients were distributed randomly, making it impossible to compare it with a control group.

Chloroquine phosphate, which has been reported to be effective in the treatment of chronic CHIKV arthritis, is also discussed.[16] However, in a clinical trial by Chopra et al.,[11] no differences were observed between the placebo group and the treatment group in CHIKV-infected patients. It should be emphasized that chloroquine exhibits antiviral and anti-inflammatory properties that deserve attention in the clinical management of some viral diseases.[16]

There is also a study by Crostein and Sunkureddi[17] that refers to colchicine, which is a drug used since the 18th century in the treatment of acute gouty arthritis and has anti-inflammatory effects. Colchicine is an antimitotic alkaloid that disrupts cytoskeletal assembly, intracellular signaling in neutrophils and inhibits neutrophil migration by decreasing the expression of neutrophil adhesion molecules. In a case report by Redel,[18] this drug was used at the dose of 0.6 mg per day in a patient with persistent arthralgia in the ankle and left wrist, with bilateral edema in the lower extremities. After 2 to 3 days, the patient had already seen resolution of the swelling and improvement of arthralgia. In two months of use, the symptoms were resolved. The patient continued to use colchicine for six months, and had no adverse events. Therefore, the case report shows that the patient was free of symptoms for eight months. Colchicine is suggested to be a therapeutic option for cases of persistent arthralgia due to CHIKV, despite treatment with non-steroidal anti-inflammatory drugs.

In addition to pharmacological treatment for rheumatic manifestations of the late phase of CHIKV, Ribeiro et al.[14] report the efficacy of ten sessions of continuous ultrasound with a frequency of 1 MHz applied once a day, from Monday to Friday, followed by infrared laser at the

dose of 4 J and 3 s per point, and TENS-burst with a pulse width of 250 uS and frequency of 2 Hz. This association showed a significant post-intervention improvement for quality of life assessed by SF-36 (Medical Outcomes Study 36) and visual analogue scale (VAS) scores. The study by Ribeiro et al.[14] is presented as a justification for the effectiveness of the physiotherapeutic treatment, since the application of continuous ultrasound transmits the heat by convection, causing an increase in blood flow through vasodilation, capillary permeability, speed of muscle contraction, nerve conduction, cellular metabolic rate, and extensibility of collagen. TENS, transcutaneous electrical current, stimulates large afferent sensory fibers that block the primary nociceptive fibers releasing endorphins and decreasing pain. The light from low-power laser therapy produces photochemical reactions within cells that activate enzymes, at the cellular level, with the ability to increase mitochondrial function and ATP synthesis, increasing cell proliferation and accelerating the healing process.[14] But, as reported in the study itself, the sessions are time-consuming, especially considering that patients usually have complaints in several joints, making the sessions longer and rendering it unfeasible to treat a larger numbers of patients. Also, the study is a case report, requiring more research with a larger sample.

Another alternative to traditional pharmacological treatment, in addition to the physiotherapeutic treatment already mentioned, is homeopathy. In a study by Wadhwani,[15] both acute and chronic phase patients were studied, focusing on the latter. Twenty (20) people with chronic chikungunya arthritis were selected and 90% of the cases achieved full recovery after the average of 32.5 days.

The treatment was done in an individualized manner, i.e. the homeopathic remedies used were not the same for the 20 patients, and included: *Lycopodium*, *Arnica montana*, *Rhus toxicodendron* followed by *Bryonia alba*, *Bryonia alba* followed by *Rhus toxicodendron*, *Ignatia amara*, *Calcarea carbonica*, *Calcarea phosphorica, Lachesis Muta*, *Natrum muriaticum*, *Phytolacca decandra* and radium bromide. Patients in this study did not return to traditional treatment, and homeopathic prescription is an alternative to be further explored.[15]

Conclusion

There was a long-term indication of the use of methotrexate and hydroxychloroquine for treatment, but they did not always resolve arthralgia in the chronic phase of CHIK. Thus, other drugs such as ribavirin and colchicine, and the maintenance of analgesics and anti-inflammatory drugs, judiciously used, have been proposed as alternatives (Figure 2). Physiotherapeutic treatment has shown some satisfactory results through electrothermal therapy. Homeopathy has also been an alternative in therapeutics (Figure 3).

But investigations must be intensified, since the literature on CHIK is still scarce. In addition, it is important

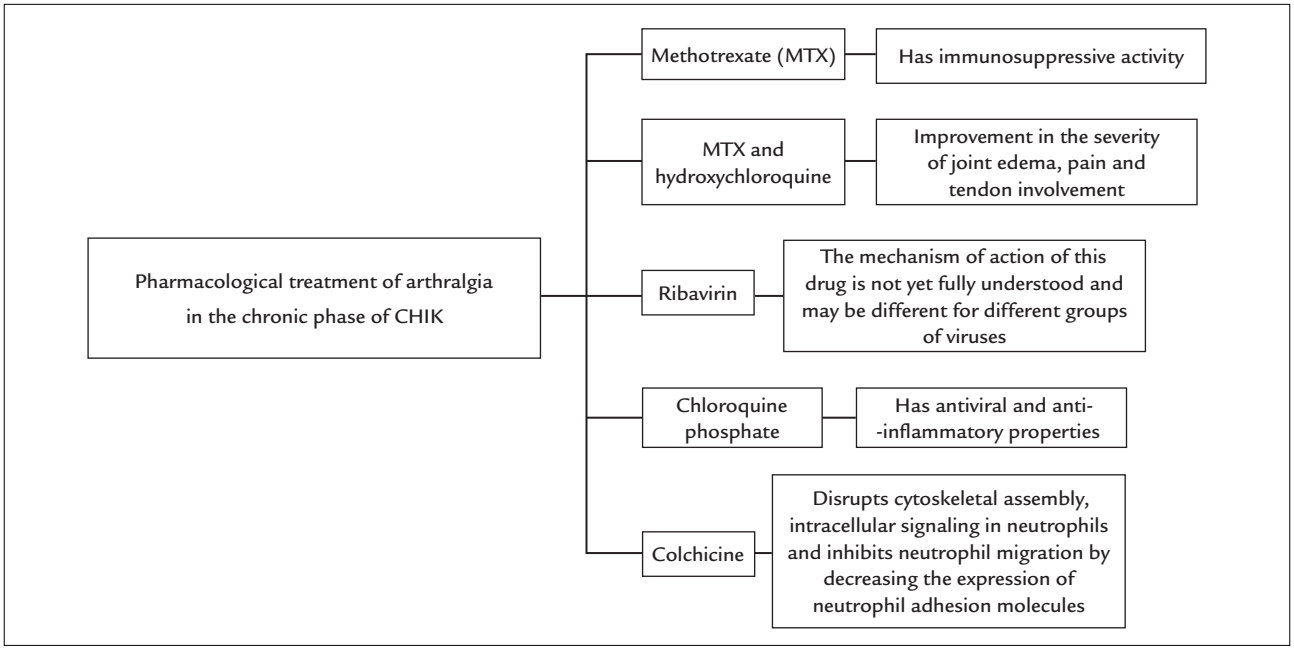

FIGURE 2 Conceptual map of pharmacological treatment of arthralgia in chikungunya.

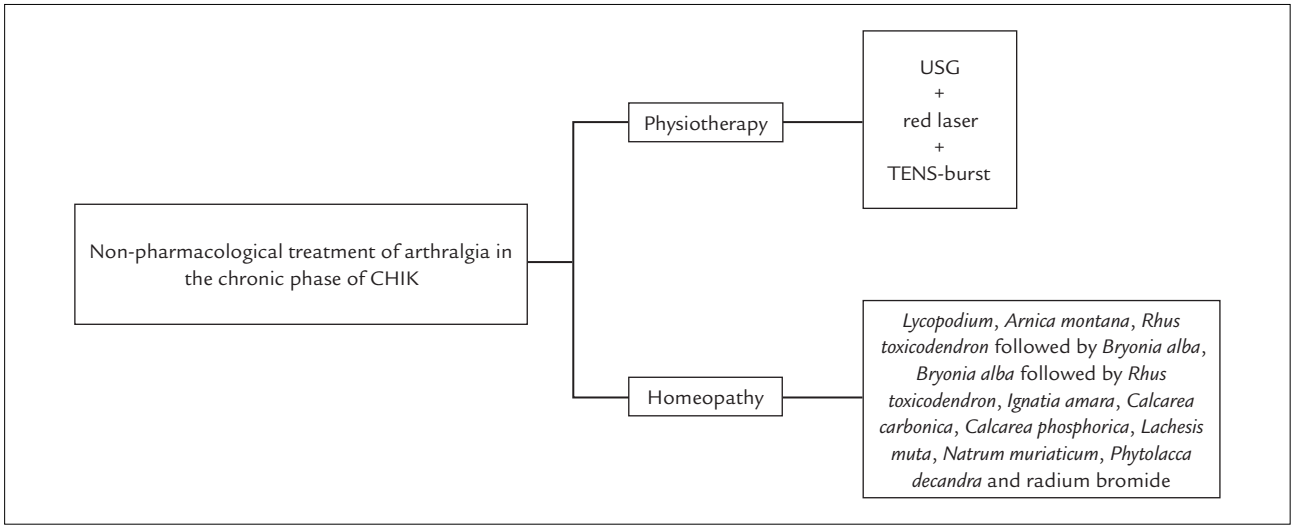

FIGURE 3 Conceptual map of non-pharmacological treatment of arthralgia in chikungunya.

to emphasize to the public the need for disease prevention, through educational campaigns and more vigorous supervision by the competent bodies.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Tratamento de artralgia crônica da chikungunya: uma revisão sistemática

Introdução: A chikungunya é uma arbovirose tropical, transmitida pela fêmea dos mosquitos *Aedes aegypti* e *Aedes albopictus*. No Brasil, existem casos relatados desde 2014. As manifestações iniciais dessa virose são: febre alta de início súbito, cefaleia, calafrios, erupções cutâneas, mialgia e dor articular intensa. Normalmente, a chikungunya apresenta as fases aguda e crônica, sendo a última caracterizada pela poliartralgia bilateral, que pode durar meses e até anos. Durante esse período, doenças autoimunes podem ser desencadeadas, tornando o quadro ainda mais complicado.
Método: Foi realizada uma revisão sistemática nos bancos de dados PubMed e Scielo em janeiro de 2017. Ensaios clínicos, coortes, casos-controle e relatos de caso foram incluídos na pesquisa. Opiniões de especialista, consensos de sociedades e revisões literárias foram critérios de exclusão. Foram avaliados estudos nas línguas inglesa, espanhola e portuguesa. Os estudos foram analisados descritivamente, e os dados agrupados, conforme semelhança metodológica.

Resultados: Foram selecionados 24 artigos; em obediência aos critérios de inclusão e exclusão, 18 foram eliminados, restando seis estudos na presente revisão: cinco ensaios clínicos e um relato de caso.
Conclusão: Quando as manifestações da chikungunya se tornam crônicas, quanto mais tempo duram, mais complicações surgem. A poliartralgia pode ser incapacitante, afastando os indivíduos das suas atividades de vida diária. Anti-inflamatórios (esteroides ou não), somados a imunossupressores, homeopatia e fisioterapia são medidas de tratamento que, conforme a literatura, têm alcançado êxito no alívio ou na extinção dos sintomas. Todavia, é fundamental que os estudos do tratamento da chikungunya sejam mais aprofundados.

Palavras-chave: Vírus Chikungunya. Artralgia. Artrite.

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Positron emission tomography/magnetic resonance imaging (PET/MRI): An update and initial experience at HC-FMUSP

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Summary

The new technology of PET/MRI is a prototype of hybrid imaging, allowing for the combination of molecular data from PET scanning and morphofunctional information derived from MRI scanning. Recent advances regarding the technical aspects of this device, especially after the development of MRI-compatible silicon photomultipliers of PET, permitted an increase in the diagnostic performance of PET/MRI translated into dose reduction and higher imaging quality. Among several clinical applications, PET/MRI gains ground initially in oncology, where MRI per se plays an essential role in the assessment of primary tumors (which is limited in the case of PET/CT), including prostate, rectal and gynecological tumors. On the other hand, the evaluation of the lungs remains an enigma although new MRI sequences are being designed to overcome this. More clinical indications of PET/MRI are seen in the fields of neurology, cardiology and inflammatory processes, and the use of PET/MRI also opens perspectives for pediatric populations as it involves very low radiation exposure. Our review aimed to highlight the current indications of PET/MRI and discuss the challenges and perspectives of PET/MRI at HC-FMUSP.

Keywords: Positron-Emission Tomography. Tomography, X-Ray Computed. Magnetic Resonance Spectroscopy. Diagnostic Imaging. Review.

Introduction

Positron emission tomography/magnetic resonance imaging (PET/MRI) stands out as the most advanced method in the field of imaging diagnosis, in addition to being one of the precursors of a new modality in the field, namely molecular imaging. Combining the molecular information made available by PET with the morphological and functional data from MRI allows for a complete and detailed assessment of the patient.

The idea underlying the development of hybrid technologies, i.e. technologies combining different diagnostic methods, was first established in 1991 in Geneva when Townsend et al. developed a PET unit with gaps between its detectors, which permitted the integration of another imaging method: as per the suggestion of Swiss cancer surgeon Rudi Egeli, computed tomography (CT) was chosen. The PET/CT concept was thus born. Nevertheless, it only became a prototype for clinical use in 1998 in Pittsburgh, offering sequential PET and CT acquisitions with a diagnostic quality. About 300 patients underwent PET/CT scans, with the promising results having encouraged the development of a PET/CT unit for commercial use. Then, in 2001, the first PET/CT unit was made available by three different manufacturers. Its success was instantaneous and significant for clinical routine, to the extent that in 2006 exclusive PET units were no longer marketed, and in 2008 more than 2,500 PET/CT units were already available worldwide.[1]

At the same time, the idea of integrating MRI into PET was also proposed. However, the technical difficulty involved is much greater, given the incompatibility of PET detectors with magnetic fields. Also in 1997, the possibility of simultaneous phantom PET and MRI acquisitions was demonstrated.[2] Only in 2006, in Germany, after the development of software that can be used to fuse PET and MRI images, the first PET/MRI prototype was developed for exclusive evaluation of the brain.[3] The PET/MRI concept for use in humans has been proven, albeit with

many technical limitations for its incorporation into clinical practice. In 2010, the first PET/MRI unit became commercially available. But as it involved the integration of PET/CT and MRI units in the same room (Philips Healthcare) or in different rooms (GE healthcare), it was thus referred to as sequential technology. In 2011, the first PET/MRI with integrated technology for simultaneous acquisitions (Siemens Healthcare) was launched for clinical use.[4] More recently, in 2014, a more modern model, also relying on an integrated, time-of-flight technology, was made available.

Shortly after its arrival in Brazil, PET/MRI was initially used for scientific purposes, especially as a research tool, and has been showing promising results in several fields, mainly oncology, neurology and cardiology. Currently included in the clinical routine at some centers and referred for some indications, PET/MRI faces encouraging prospect as the future of imaging diagnosis, for it allows a noninvasive, functional assessment coupled to a high morphological resolution.

Technical aspects

General technical concepts

The combination of PET and MRI in a single unit that permits simultaneous acquisitions, although intuitively simple, is technically much more complex than it appears to be. What made this integration impossible for many years was the incompatibility between MRI's strong magnetic field and PET's photomultiplier tubes. The initial solutions for this problem proposed that sequential acquisitions should be made. One of such solutions was the PET/CT and MRI trimodality system (General Electrics – GE), where the patient undergoes sequential MRI and PET/CT scans (following no previously defined order) in separate rooms. This system uses a stretcher with a floating device for patient transferring that prevents him or her from moving about, which therefore allows for the subsequent and accurate fusion of images.[5] After that, a sequential PET/MRI system was launched, but it comprised a single unit (Ingenuity – Philips) where PET detectors are separated from the magnet, linked by a connecting table that transports the patient in order to acquire the images from each component separately.[6] Although more realistic, this latter solution remains sequential in nature and loses a sense of simultaneity as far as acquisition is concerned.

It was necessary to create a PET detector with MRI-compatible semiconductors in order to make a true architectural fusion possible. The first solution for this great challenge came about via avalanche photodiode detectors (APD), which resist magnetic fields up to 9.4 Tesla. It was with this technology that the first commercial simultaneous PET/MRI unit (Biograph – Siemens) was launched.[7] Subsequently, a new technology of magnetic-field-compatible PET detectors was developed, i.e. detectors with silicon photomultipliers (SiPM).[8] Such technology was implemented in the market as the second simultaneous PET/MRI machine (Signa – GE) to be made available. The detectors with SiPM displayed greater detection sensitivity, hence promising clinical gains such as dose reduction with better image quality and thus allowing greater flexibility of PET/MRI protocols.[9-11] These are the two PET/MRI machines available on the market offering simultaneous acquisition. They are equipped with 3-Tesla magnetic field MR, with one of them relying on time-of-flight (TOF) technology.

Following the great challenge of integrating the PET detector crystals into a 3T magnetic field, the next challenge was a need for a very accurate correction of the detected gamma rays due to the attenuation correction (AC) caused by different types of tissues.

MR-based attenuation correction (MRAC) is broadly based on a tissue classification using the T1-weighted MRI sequence (T1w) Dixon,[11-13] differently from the tissue density on which PET/CT AC is based (CTAC). The post-processed Dixon generates four distinct sequences, "Water only" (or "Water"), "Fat only" (or "Fat"), "In-phase" (or "IP"); and "Out-of-phase" (or "OP"). In combining such tissue information, an algorithm in the unit makes a tissue classification, namely air, lung, fat and soft tissue. MRAC is generated by means of this tissue stratification. This technique was already validated by some studies for clinical use in the two modalities of simultaneous PET/MRI units, either with or without time-of-flight (TOF) technology.[14-17] Another method used for generating the MRI in the skull portion involves an atlas. This technique is based on MRI recognition of anatomical patterns, generating what would be a pseudo-CT scan of the skull and an attenuation correction map.[18]

One problem that both current MRAC techniques generate is that they do not correctly factor the "bone" for that AC, which causes PET/MRI to still attract ongoing criticism regarding standard uptake value (SUV) quantification of bone lesions. To this end, recent MR-sequence studies with the use of zero echo time (ZET) showed that this technique can overcome the current technical limitation, thereby generating a more accurate and reliable quantitative MRAC.[19,20]

Technical concepts of image acquisition

PET/MRI acquisition is said to be truly simultaneous when PET data are acquired while MRI sequences are being performed in the same region. As is the case with other sectional methods, it all begins with the acquisition of MRI localizer images (the equivalent to a CT scout scan in PET/CT), which aims at defining the imaging coverage area and serves as a basis for programming both the PET and MRI acquisition sequences. The region of the body to be scanned is divided into smaller portions called "beds" according to the size of the PET detector ring. The axial length of the bed in the latest generation PET/MRI scanner is 25 cm, with an axial bed position overlap of 23%. Depending on the patient's body length in a full-body study (head to thigh), usually 5-7 beds are acquired. PET imaging is acquired in sequential blocks of bed positions, from cranial to caudal, and it is necessary to define beforehand how many beds are to be acquired and the amount of time acquiring data at each single bed position. The acquisition time of each bed is similar to that in PET/CT, between 2-4 minutes, as recommended by international guidelines, but modifiable in accordance with the desired protocol. The MRI sequences are then programmed for each bed in accordance with the protocol established for the context. Therefore, if longer MRI sequences within a particular segment of the body are required, it is important to program this in advance in order for PET time to be proportional.

When PET acquisition is initiated, a sequence for optimizing magnetic field homogeneity is quickly acquired for each bed. The T1 Dixon referring to MRAC is sequentially acquired. Altogether, the whole process takes less than 30 seconds. Thereafter, the remaining anatomical and functional sequences follow in accordance with the stipulated protocol.

Once the simultaneous acquisition is completed, it is possible to program new segmental acquisitions of some region of interest, either simultaneous PET/MRI or MRI – only scans, for instance, post-contrast sequences, without necessarily involving PET acquisition. When everything is finalized, then it is possible to proceed with post-processing and generating the fusion of PET data, MRAC data and other desired sequences, which are then sent for analysis.

Clinical applications

Oncology

Head and neck neoplasms

MRI plays an already well-established role in local staging of head and neck neoplasms, especially in preoperatively assessing the tumor's relationship with adjacent structures, detecting infiltration of the prevertebral fascia or perineural dissemination. Furthermore, MRI is superior to CT in detecting occult primary neoplastic sites in patients with cervical lymph node metastases. With regard to imaging quality, besides MRI's well-known superiority in spatial resolution, it is associated with lower prevalence of artifacts stemming from metal dental artifacts, which usually hampers assessment when using CT.[21] The same applies to comparing PET/MRI versus PET/CT, favoring the greater sensitivity of the former in T staging, detecting occult primary neoplastic sites, and evaluating tumors of the oropharynx and oral cavity.

In relation to N and M staging, there is an important limitation of the imaging methods with respect to diagnostic accuracy. CT and MRI basically take into account morphological alterations or, occasionally, biological changes, such as increased vascularization and hypercellularity detected by the functional sequences of diffusion and perfusion MRI, respectively.[22,23] PET imaging, despite the spatial limitation of PET methods that does not allow the diagnosis of metastases of less than 5 mm, contributes with an evaluation at the molecular level, either relative to glycolytic metabolism, with the widely available FDG (fluorodeoxyglucose), or hypoxia, with the less known 18F-FMISO and 18F-FAZA. Therefore, the synergism of hybrid imaging methods, especially PET/MRI, can minimize such limitation by combining morphological, biological/functional and molecular information.[24,25] In the evaluation of metastatic disease, PET/CT does have a relative advantage, given the higher prevalence of mediastinal lung and lymph node metastases, well-demonstrated by PET/CT, as compared to PET/MRI, which still needs to overcome some limitations regarding pulmonary parenchymal evaluation. The importance of whole-body evaluation is also due to the high prevalence (approximately 15%) of synchronous neoplasms, mainly in the esophagus and lung.[21]

In the evaluation of response to therapy, PET/MRI also displays diagnostic accuracy similar to that of PET/CT, with the additional ability to specify additional findings possibly related to the disease. In a post-treatment context, changes induced by the treatment, mainly surgery and radiotherapy, lead to the distortion of the anatomy, which hinders the differentiation between residual and relapsed disease. There is no consensus regarding the ideal timing for conducting a PET scan following the end of treatment. Nevertheless, an interval of at least 8 weeks is recommended in an attempt to minimize post-therapy inflammatory changes, hence avoiding false positives. For follow-up, as recurrence usually occurs within two years after

treatment, at least another two additional annual hybrid PET studies (at 12 and 24 months) are recommended.

PET/MRI can also potentially assist in the planning of radiotherapy. The development of new units compatible with PET and MRI drives the use of PET/MRI for delineating the primary tumor with more consistent data than employing MRI alone.[26,27]

As far as quantification by PET/MRI is concerned, SUVmax measurements are comparable to those achieved by PET/CT and applicable to studies in humans.[28,29] Semiquantitative data, such as standard uptake value (SUV), total lesion glycolysis (TLG) and tumor metabolic volume (TMV), made available by PET, can be used as prognostic predictors, identifying patients at greatest risk of therapeutic failure who could benefit from a more aggressive therapeutic approach and server as markers of survival.[30-32]

Thus, PET/MRI could potentially replace PET/CT in the evaluation of head and neck tumors, providing the data required for staging, evaluating treatment response, planning radiotherapy, and predicting the prognosis in one single examination.

Thoracic neoplasms

Among the thoracic neoplasms with potential clinical application, the ones that stand out the most are lung cancer, mesothelioma, and breast cancer.

Lung cancer

PET/CT constitutes the reference imaging method for the staging of non-small cell lung cancer, offering high diagnostic accuracy for the detection and delineation of primary tumors; CT scans are acquired during deep inspiration. Furthermore, the ability to detect regional lymph node disease and distant metastases gives PET/CT gold standard status in evaluating lung neoplasms.[33]

Recent studies indicate similar diagnostic accuracy of PET/MRI in evaluating lung cancer, despite its low ability to detect lung nodules by using conventional sequences, especially if those are smaller than 1.0 cm.[34] Still, its multiparametric PET/MRI evaluation capability allows this occasional limitation to be overcome. One of its potentials relates to the use of T2-weighted high-resolution sequences, which aid in characterizing thoracic wall invasion. In addition, the integration of diffusion, ultra-short echo-time sequences allows for a better evaluation of the pulmonary parenchym.[35-37] For the assessment of N and M staging, PET imaging has a high negative predictive value, even though the positive predictive value is low.[38] The incorporation of new sequences and radiopharmaceuticals, such as FLT, a marker of cell proliferation, can be determinant when assessing lung neoplasms, especially so with regard to evaluating treatment response and prognostic prediction.[39,40]

Breast cancer

The most prevalent malignant neoplasm in women is also associated with a high mortality rate. Usually, breast cancer staging is performed with MRI for local evaluation and, in more advanced cases, with PET/CT for detection of lymph node disease and, mainly, distant metastases. FDG PET/MRI may offer benefits for both local and systemic staging.[41] The first advantage is a lower radiation exposure (about 50% of the PET/CT dose). The second one refers to the detection rate of metastatic lesions, i.e. PET/MRI's greater sensitivity when associated with FDG makes it more suitable for hepatic, cerebral and bone evaluation, but still limited for the identification of lung lesions.[42,43]

A recent study evaluated the clinical usefulness of FDG PET/MRI in breast cancer staging as compared to either PET or MRI alone. MRI showed greater detectability of metastatic lesions, and PET/MRI was responsible for changing the course of medical action in one third of the 36 patients who were analyzed.[44] Another study demonstrated PET/MRI and MRI to have similar performances, with high specificity when characterizing regional lymph node disease.[45] In addition, the possibility of using semiquantitative PET and MRI parameters can help characterize the expression of tumor biological factors, with a good correlation with intratumoral heterogeneity, Ki-67, triple-negative breast cancer and lymphovascular invasion.[46]

Given the importance of breast cancer on a molecular level, advancements in the development of new radiopharmaceuticals can be potentially useful in evaluating breast cancer. This applies to PET/CT with [^{18}F]NaF (Fluoride) which, in combination with FDG PET/CT, showed diagnostic accuracy superior to that of whole-body MRI and bone scintigraphy with MDP.[47] Another radiopharmaceutical having potential applications in breast cancer is [^{18}F] Fluoroestradiol (FES), an estrogen analog with a high correlation with tumor expression of estrogen in both primary tumors and metastases.[48] FES PET shows very promising results, with a significant clinical impact (responsible for changes in the medical course of action in 48% of patients with a clinical dilemma)[49], and high conspicuity in detecting heterogeneity in estrogen receptor expression. It can potentially have a role as a predictor of treatment response, since lesions that uptake this radiopharmaceutical tend to respond to anti-hormonal therapy.[49-51]

Abdominal neoplasms

The liver is one of the organs most commonly affected by neoplasms, both primary (hepatocarcinoma and cholangiocarcinoma) and secondary (metastases, mainly colorectal carcinoma). For this reason, hepatic evaluation emerges as one of the main PET/MRI niches, capable of coupling the evaluation with functional sequences (diffusion and perfusion) and the use of a hepatobiliary contrast agent with the molecular information derived from PET. In doing so, it boosts diagnostic capacity. Furthermore, the evaluation of pancreatic neoplasms, including adenocarcinoma and neuroendocrine tumors, and pelvic neoplasms, such as adenocarcinoma of the prostate and gynecological malignancies, faces interesting prospects with PET/MRI.

Liver

The evaluation of the liver comprises one of the promising clinical applications of PET/MRI, given the high prevalence of hepatic metastases. Among the reasons for that are the excellent diagnostic performance of MRI when using functional sequences (diffusion and perfusion) and hepatobiliary contrast, and the superb detection ability of FDG PET to provide molecular information.

Some studies compared PET/MRI and PET/CT suitability for hepatic evaluation, with interesting results. Reiner et al.[52] demonstrated that PET/MRI, with T1-weighted and T2-weighted sequences alone and without the injection of a paramagnetic contrast agent, showed a diagnostic accuracy similar to that of contrast PET/CT relative to lesion detection. More recently, Lee et al.[53] demonstrated that PET/MRI with a hepatospecific contrast media had a diagnostic performance that was significantly superior to those of CT and PET. This also indicates the possibility of using PET parameters as prognostic predictors of lower survival in the subgroup of high-uptake patients following neoadjuvant chemotherapy. PET/MRI with other radiopharmaceuticals, such as [^{68}Ga]DOTA-TOC, is also highly suitable for diagnosing hepatic lesions, especially when combined with a hepatobiliary contrast agent. This makes for the combination of MRI detection with PET molecular information, i.e. somatostatin receptor expression in this specific case, thereby allowing the identification of patients who could benefit from peptide therapy.[54]

There is, therefore, an excellent niche for PET/MRI in liver evaluation, especially for evaluating liver metastases, with an excellent performance relative to detection, characterization and prognostic prediction.

Pancreas

Recently, the use of PET/CT for staging pancreatic adenocarcinoma was shown to be an excellent complementary method to the already well-established contrast CT. The results were obtained in a prospective, multicenter study conducted in England that evaluated the use of PET/CT in the diagnosis, staging and management of patients with suspected pancreatic cancer; staging required correcting in 14% of the cases. Comparatively, 45% of the 550 patients assessed with contrast CT, in turn, involved changes in the medical course of action. Furthermore, it was proven to be cost-effective, being especially useful in preventing unnecessary surgery in 20% of the patients.[55]

Few studies assessed the clinical use of PET/MRI in pancreatic evaluation. Chen et al.[56] correlated clinical stage and prognoses for pancreatic and periampullary tumors with imaging biomarkers, diffusion by using MR spectroscopy, and glycolytic metabolism by using PET. They concluded that PET/MRI imaging biomarkers can predict clinical stage and progression-free survival in this group of patients.

Neuroendocrine tumors (NETs)

Gastroenteropancreatic NETs constitute the most common presentation of this tumor type and are often associated with lymph node and liver metastases. Most NETs have low FDG avidity and a high expression of somatostatin receptors, which depends directly on the cell proliferation index (the higher the Ki-67 and, consequently, the lower the tumor differentiation, the higher the FDG uptake and the lower the expression of somatostatin receptors).[54] The use of PET radiopharmaceuticals analogous to somatostatin and the different types of [^{68}Ga]DOTA have a promising role in evaluating NETs: they can be used in PET/MRI scans and their detectability is similar to PET/CT.[57] A recent study compared PET/MRI with both DWI and MRI using a hepatospecific contrast agent. Both methods were shown to be highly accurate, with DWI having some limitations when differentiating malignant from benign lesions.[58] Hence, the combination of MRI functional sequences, especially diffusion ones, with the PET information regarding receptor expression may represent the best imaging method in evaluating NETs.

Prostate

MRI represents the best method for diagnosing and staging prostate cancer. This, not only due to a better soft tissue contrast, but mainly because of the functional sequence values, such as diffusion and perfusion.[59]

The images obtained from FDG PET have low sensitivity for prostate cancer, since uptake is minimal in early and well-differentiated tumors and urinary excretion causes artifacts that undermine an appropriate analysis. Therefore, FDG PET/CT is a limited method for the evaluation of patients with prostate cancer. Other radiopharmaceuticals such as ^{68}Ga-PSMA, ^{18}F-Choline and ^{11}C-Choline are best suited for staging and evaluating the biochemical recurrence of prostatic adenocarcinoma.[60]

PET/MRI with diffusion is considered a promising tool in the pre-therapeutic evaluation of prostate tumors, allowing for the acquisition of detailed anatomic data in conjunction with molecular parameters. Choline, either ^{18}F- or ^{11}C-marked, is the most studied tracer to date. However, ^{68}Ga-PSMA has been gaining strength due to its high diagnostic specificity. Choline PET/MRI can perform staging more effectively than MRI alone does – in terms of both primary tumor characterization and how it relates to adjacent structures, especially the prostatic capsule, and the possibility of guiding biopsy to an area of greater suspicion in clinically significant neoplasms. This reduces sampling errors of occasional random biopsies and positively influences therapeutic management.[61,62] Yet, caution should be taken with false positives related to the use of choline, such as increased uptake in cases of benign prostatic hyperplasia and other findings not related to the disease, such as inflammatory mediastinal lymph nodes.[63]

In the context of tumor recurrence, usually detected as biochemical recurrence due to increased serum levels of prostate specific antigen (PSA), the evaluation using conventional imaging methods has low sensitivity for detecting disease outbreaks. ^{68}Ga-PSMA stands out precisely as the best option for overcoming such limitations and identifying biochemically recurrent prostate cancer metastases. PET/MRI with ^{68}Ga-PSMA may be the only imaging modality for conducting this evaluation, thereby reducing the number of false positives.[64-66]

Thus, it is believed that PET/MRI with non-FDG radiopharmaceuticals, especially ^{68}Ga-PSMA, will represent the modality of choice for evaluating prostate cancer, both for staging it and carrying out a post-treatment evaluation, probably dispensing with any other methods.

Uterus and ovaries

MRI plays a major role in the local staging of gynecological malignancies, especially those of the uterine cervix, endometrium and ovaries. On the other hand, FDG PET/CT has greater accuracy in evaluating regional and distant staging, since it adds molecular parameters to essentially morphological alterations.[67,68] Hence, PET/MRI might be considered a superior method for detecting, staging and restaging gynecologic malignancies, whose results are similar to those obtained from multiple examinations, all added up.[69]

T2-weighted sequences appropriately evaluate myometrial invasion in patients with endometrial cancer, cervical neoplasm infiltration of parametria and the lateral pelvic wall, and improved detection of ovarian and uterine lesions.[69-71] Detectability of lymph node disease is enhanced, which allows the selection of patients needing lymphadenectomy, as is accuracy in identifying distant metastases, notably peritoneum, liver and bone metastases.[72,73]

Lymphomas

FDG PET/CT can be considered one of the pillars in the staging and evaluation of treatment response in high-grade lymphomas, known as Hodgkin's disease and diffuse large B-cell non-Hodgkin's lymphoma. The globally accepted guidelines for the assessment of lymphoproliferative disease recommend the use of PET/CT for the staging, post-treatment control and restaging of the disease, which, in these cases, leads to high exposure to ionizing radiation.[74]

Hence, PET/MRI constitutes an immediate advantage for the evaluation of this group of patients, represented by young patients with a high chance of cure. This method involves less exposure to radiation, which therefore minimizes its cumulative effects.[75]

Some studies with essentially preliminary data suggest PET/MRI could possibly replace PET/CT, while keeping similar diagnostic accuracy and good image quality.[76,77] Diffusion has been widely investigated as an alternative to PET imaging in the evaluation of patients with lymphoma. However, DWI's high sensitivity alone is not enough to match the excellent accuracy of FDG PET. Still, it may enhance diagnostic performance when added to the PET/MRI protocol.[78,79]

Neurology

Cerebral evaluation is the most promising field for PET/MRI. Scholars say that the brain, from a functional standpoint, never remains the same along different moments in time. Thus, simultaneous PET and MRI acquisition permits the multiparametric evaluation (structural, functional, molecular) of different neurological pathologies, such as neurodegenerative dementias and disorders, epilepsy, neoplasms and psychiatric conditions.[80]

In addition to providing high-resolution morphological data, several other MRI properties allow for the evaluation of different brain processes: vascular anatomy (angioresonance), tissue kinetics (diffusion), cerebral

perfusion patterns (perfusion), tissue metabolite concentration (spectroscopy), regional cerebral functional activation (functional MRI), brain fiber tract analysis (diffusion tensor imaging – DTI), oxygen consumption patterns (BOLD sequence), among others.[81]

PET also encompasses a wide range of molecular processes that can be evaluated: brain flow ($H_2{}^{15}O$), metabolism (FDG), blood volume ($C^{15}O$), oxygen consumption (^{15}O), vascular permeability (labeled amino acids), nucleic acid synthesis (FLT), neurotransmitter evaluation (DOPA), receptor evaluation (raclopride), angiogenesis (^{18}F-RGB) are just a few examples.[82]

This means that there can be a real-time image of a multitude of complementary information. Nevertheless, it is essential to define what is really necessary and what could be just redundant. New techniques may demonstrate a local functional change in degenerative or autoimmune diseases even before the onset of clinical manifestations. It is also possible to better understand the mechanism of brain structures playing an important role in behavior and cognition, as well as other interesting questions that, until then, could only be evaluated in vitro or with histological studies.[83]

Cardiovascular

Hybrid PET/MRI raised new prospects for cardiovascular applications. PET/CT offered advances in the combined anatomical and functional evaluation of coronary heart disease and alterations in cardiac function, whereas MRI, in the other hand, with its high-definition and dynamic images, provides complementary information, such as those on myocardial blood flow, influencing.[84]

MRI offers a number of advantages over conventional methods such as CT and/or SPECT, some of which stand out: good soft-tissue contrast coupled with a high spatial resolution, myocardial perfusion evaluation, cardiac volume and ventricular function calculation, identification of valvular morphology and the ability to differentiate scars from viable myocardial tissue after infarction. PET, in turn, is the reference examination for detecting hemodynamically significant stenosis. Additionally, it can also be used to evaluate myocardial blood flow in rest and stress, and also to estimate coronary flow reserve.

Thus, the complementarity of PET/MRI information seems to be very promising for cardiovascular evaluation. More importantly, such information is readily applicable to the detection and characterization of coronary artery disease, evaluation of cardiomyopathies of different etiologies (ischemic, inflammatory/infiltrative) and study of myocardial viability. Furthermore, PET/MRI could be used to evaluate inflammatory response following myocardial infarction, atherosclerosis, thus identifying vulnerable plaques. It could even be used in stem cell therapy and characterization of neoangiogenesis.[85]

New non-FDG radiopharmaceuticals could assist in adding to the complementarity of PET information, such as the myocardial perfusion tracers currently being investigated, namely ^{13}N-ammonia, ^{15}O-water, ^{82}Rb and ^{18}F-flurpiridaz.[84,85]

Thus, fusion of the most advanced methods for cardiac evaluation, i.e. PET and MRI, offers the best of the two modalities in one same procedure. Despite the need to establish its cost-effectiveness, PET/MRI could be the only method for cardiac evaluation allowing for a more complete evaluation of the main cardiac diseases and a significant impact on the therapeutic course of action.

Inflammation/Infection

PET/MRI offers the opportunity to evaluate several inflammatory processes, whose pathophysiology involves infiltration of immune-mediated cells, increased blood flow and capillary permeability, and transudation of proteins to the region involved.

In addition to its excellent soft-tissue contrast, MRI strengthens the analysis with the availability of sequences such as diffusion, which indirectly measures increases in tissue cellularity. FDG PET, on the other hand, permits the evaluation of processes with increased glucose consumption in cell recruitment of inflammatory processes.[86]

In diseases of the musculoskeletal system, FDG PET is highly sensitive in detecting joint changes, related both to overload (degeneration) and synovial inflammation. With MRI, the morphological analysis of structures such as bone marrow, muscles, tendons, ligaments, cartilage, joint capsule and fat becomes extremely viable. Thus, FDG PET/MRI permits the detection and characterization of diseases such as osteomyelitis, diabetic foot and rheumatoid arthritis.[87,88]

In the evaluation of inflammatory bowel disease, PET/MRI showed promising results when differentiating inflammatory from fibrotic stenoses in patients with Crohn's disease. In addition to the simultaneous acquisition of PET/MRI (not feasible in the sequential acquisition of PET/CT, which impairs the evaluation of intestinal loops with constant peristalsis), information such as location of different lesions in the gastrointestinal tract, detection of extra-luminal disease, and differentiation of fibrotic changes and inflammatory activity are essential for the proper clinical management of patients.[89]

More recently, data from a preclinical study showed the possibility of using PET/MRI with a specific radio-

pharmaceutical, ^{64}Cu-NODAGA, as a non-invasive, rapid, sensitive and specific method for detecting *Yersinia enterocolitica* infection. This approach was referred to as immunoPET and raised the prospect of using the method to detect different pathogens.[90]

Pediatrics

One of PET/MRI's immediate advantages is the lower exposure to ionizing radiation, with data ranging from 50 to 75% reduction relative to PET/CT. This becomes even more relevant when multiple scans are requested, as is the case with tumors submitted to different therapies. This aspect alone would have a high enough impact so as to guarantee the use of PET/MRI as a method of choice in the pediatric population.[91-93]

As an additional advantage, PET/MRI also allows for multiparametric characterization of some pathologies, thereby allowing a decline in the number of tests that need to be performed.

Among the most promising fields, PET/MRI can be used in children, oncology and neurology do stand out as the main ones. The evaluation of lymphomas, primary bone tumors, sarcomas, neuroblastomas and NETs are some of the potential clinical indications. While, in neurology, the detection of epileptogenic focus and brain tumors stand out as major points of interest. In addition to those, several other diseases may benefit from the use of PET/MRI, such as inflammatory rheumatic processes.[91,92]

The HC-FMUSP experience

The HC-FMUSP (Hospital das Clínicas, Faculty of Medicine, University of São Paulo) was the first public hospital in Brazil to use the most advanced hybrid imaging technology as it purchased a PET/MRI unit which was made available both as a research tool and for clinical use at its Nuclear Medicine Service at the Radiology Institute.

The unit is a Signa PET/MRI, a state-of-the-art GE Healthcare system (Waukesha, USA), which integrates a 3.0T MRI with a PET that fully relies on high-resolution, high-sensitivity simultaneous time-of-flight technology (its sensitivity is about three times that of PET/CT).

Having started to operate on 26 April, 2016, the PET/MRI unit at HC-FMUSP has already performed a total of 30 scans for different indications, especially those in oncology (83.3%), neurology (6.7%) and inflammatory process (6.7%) using two radiopharmaceuticals, ^{18}F-FDG (80%) and ^{68}Ga-DOTATOC (20%). Of the 30 patients, 18 were female (60%), with a mean age of 48.6 years (13-76 years).

The scan protocol included the T1 LAVA, DWI and T2 SSFSE sequences and, in selected cases, T2 PROPELLER, T2 STIR and T2 CUBE, with a mean duration of 50.3 minutes (as compared to a mean of 25.1 minutes for PET/CT).

A comparison was drawn between PET/MRI and the PET/CT, both of which were performed sequentially, relative to semiquantitative data (SUVmax from PET/MRI vs. PET/CT SUVmax). The clinical impact of PET/MRI was categorized as: 0, with no information added to PET/CT; 1, addition of information without clinical/oncological relevance; and 2, addition of information with oncological/therapeutic relevance.

With respect to semiquantitative data, SUVmax in PET/CT scans was 7.4 (ranging from 2.2 to 18.7) and PET/MRI scans was 10.9 (ranging from of 2.1 to 77.8). For malignant lesions, the mean late SUVmax (obtained from PET/MRI) was 82% higher than the early one (measured from PET/CT); whereas, for benign lesions, the mean late SUVmax was 19% lower. PET/MRI exhibited a clinical impact having potential therapeutic relevance in 10% of the cases and added information without potential therapeutic relevance in 53.3% of the studies.

A selection with the main clinical cases studied with PET/MRI is illustrated in Figures 1 to 5, specifying the respective clinical impact as compared to PET/CT.

The installation of the PET/MRI unit raised new prospects in the field of advanced research, both basic and clinical research, with the development of at least six research projects already approved by the Research Ethics Committee. They involve external funding and some of them are already underway. They encompass the most different fields, some of which stand out, namely: a) oncology: neoplasms of the rectum, breast, prostate; b) neurology: traumatic brain injury, dementia, multiple sclerosis; and c) inflammation: inflammatory bowel disease. Furthermore, different radiopharmaceuticals are being used and produced in the radiopharmaceutical division at HC-FMUSP in an attempt to foster the development of research, such as ^{18}F-FDG (a marker of glycolytic metabolism), ^{18}F-NaF (a marker of osteoblastic activity), ^{18}F-FES (an estrogen analog), ^{68}Ga-PSMA (a specific antigen from the prostatic membrane), ^{68}Ga--DOTATATE (a somatostatin analogue), ^{11}C-PK11195 (a neuroinflammation marker) and ^{11}C-PIB (a marker of beta-amyloid plaques, used in the investigation of Alzheimer's disease).

Hence, it can be noticed that the PET/MRI unit at HC-FMUSP faces the prospect of a promising future in the institution. It will be used both in research purposes, which involve a range of prospective projects and development of different radiopharmaceuticals, and in translation into clinical routine.

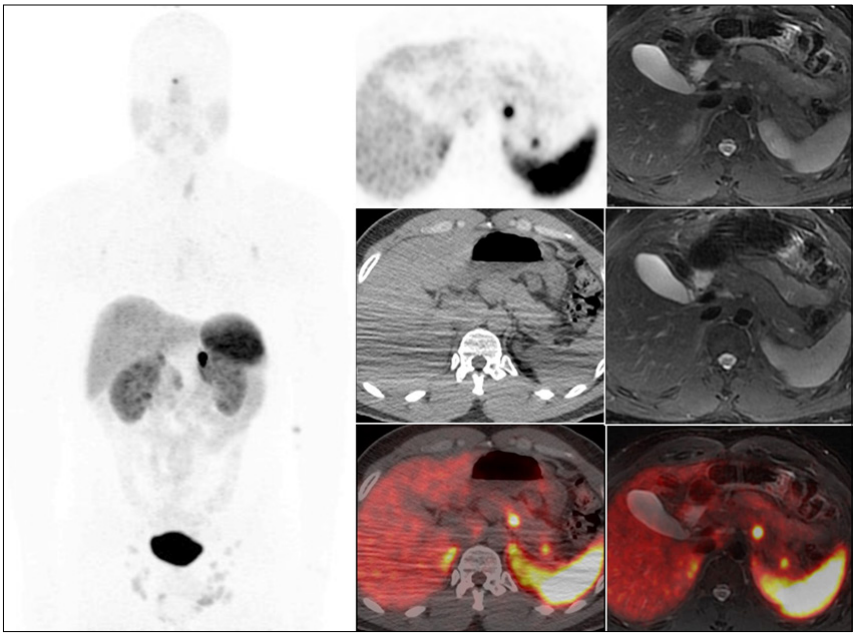

FIGURE 1 Coronal MIP PET and axial slices of T2-weighted PET/CT and PET/MRI sequences. A 35-year-old male patient with a family history of NEM1 syndrome underwent abdominal CT scan, which revealed a para-aortic nodule. He also underwent a MIBG scintigraphy yielding a negative result. A ^{68}Ga-DOTA PET scan was requested for investigation of neuroendocrine tumor. The patient had a history of thyroidectomy and pituitary hyperplasia. ^{68}Ga-DOTATATO PET/CT and PET/MR images revealed: focal areas in the body and tail of the pancreas, uncorrelated to CT images and characterized as nodules on MRI with high T2 signal and diffusion restriction. This case demonstrates the superiority compared to MRI soft tissue contrast.

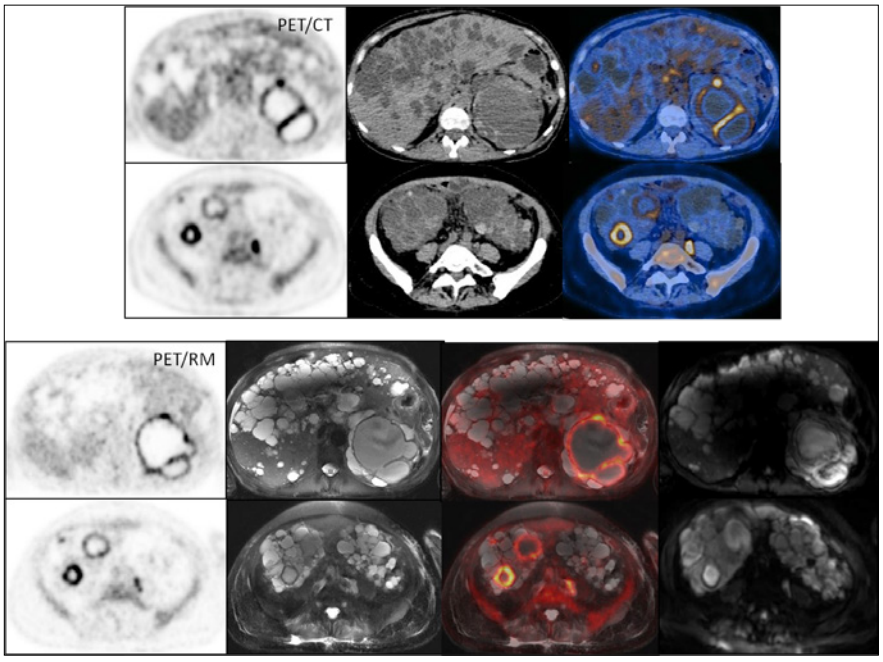

FIGURE 2 PET/CT (top) and PET/MRI (bottom) showing axial slices with T2-weighted sequences and diffusion. A female patient with polycystic kidneys and pain on palpation of the left flank, undergoing investigation due to fever of unknown origin. The PET/CT and PET/MRI studies showed an increase in glycolytic metabolism and diffusion restriction in multiple bilateral renal cysts, suggestive of an active inflammatory/infeccious processes. The case demonstrates the possibility of correlating the molecular information of the PET (glycolytic metabolism of the inflammatory process) with data from functional MRI sequences, namely diffusion (increased cellularity also related to the inflammatory process), increasing the diagnostic conspicuity.

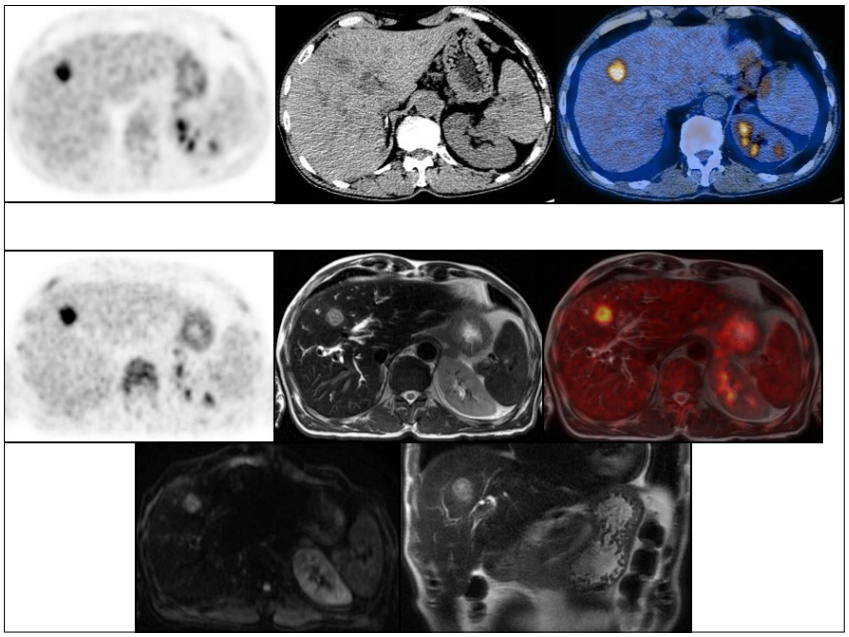

FIGURE 3 PET/CT (top) and PET/MRI (bottom) showing axial slices with T2-weighted sequences and diffusion. Evaluation after chemotherapy for diffuse lymphoma of large B cells. History of right nephrectomy due to lithiasis 4 months before the exam. History of a drain placed in the biliary tract due to choledocholithiasis, repositioned 2 weeks before the exam. A PET/MRI scan allows for a more detailed evaluation of the hepatic lesion seen on a PET/CT and considered suspicious for neoplastic process. On PET/MRI, the hepatic lesion is characterized by thick and irregular walls, with a halo of edema and diffusion restriction, being more probably related to the inflammatory/infectious process. The case demonstrates the superiority of PET/MRI in the evaluation of hepatic lesions.

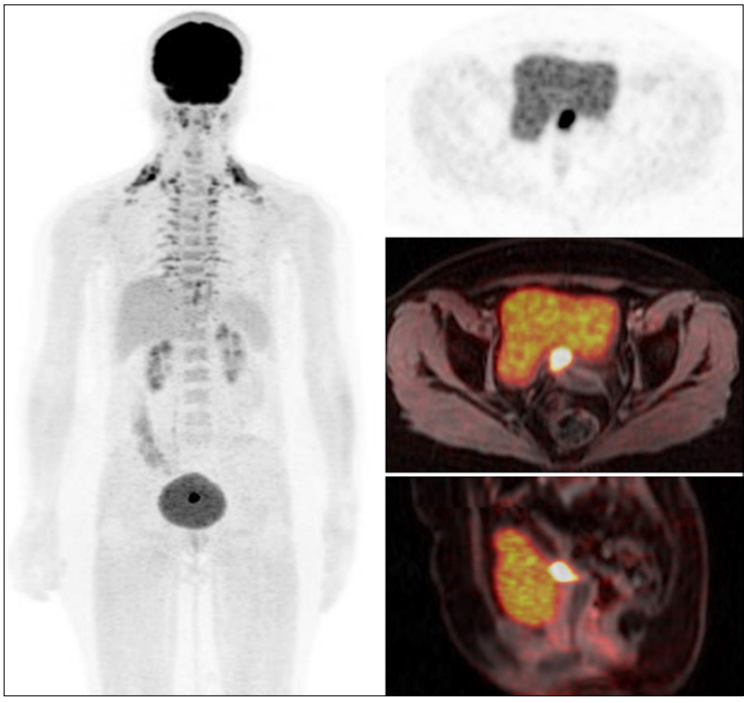

FIGURE 4 Coronal MIP PET and axial slices of T1-weighted PET/MRI sequences. A 46-year-old female patient with a history of cervical cancer treated with surgery and radiation therapy, underwent local recurrence investigation. A PET/MRI scan shows local recurrence in irregular lesions with increased glycolytic metabolism in the retrovesical region, near the vaginal dome. The case shows a potential benefit of using PET/MRI for the evaluation of gynecological neoplasias.

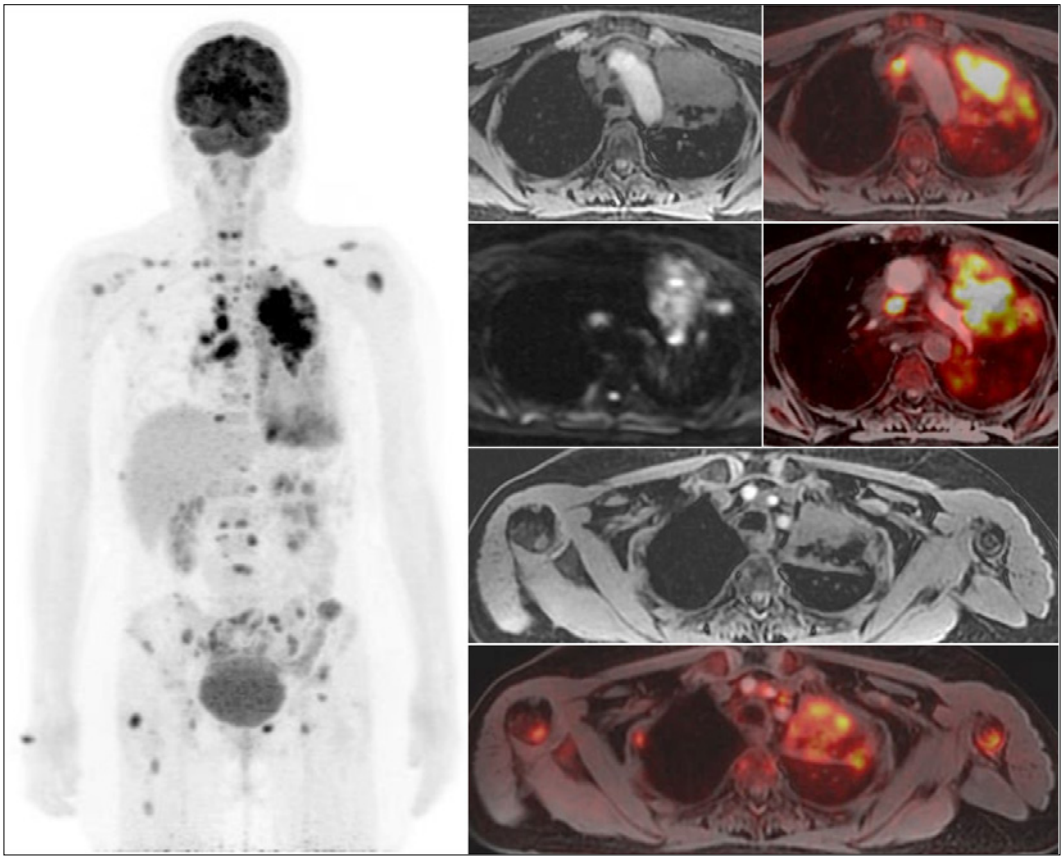

FIGURE 5 Coronal MIP PET and axial slices of T1-weighted PET/MRI sequences and diffusion. Staging of non-small cell lung adenocarcinoma. A PET/MRI scan shows the primary pulmonary mass in the left upper lobe, with signs of thoracic wall infiltration (cortical in the second rib) with bilateral mediastinal lymph node metastases and multiple sparse bone metastases, as well as good morphological correlation in the MR component. This case shows the excellent performance of PET/MRI for staging of lung neoplasia, despite the greater diagnostic capacity of CT in parenchymal evaluations.

Conclusion

Simultaneous PET/MRI, although widely used as a research tool, could potentially be used in clinical translation as the diagnostic modality of choice in different pathologies.

In oncology, its highest volume niche, the neoplasms that currently require evaluation by PET/CT and MRI can be naturally transferred to PET/MRI. The advantages far exceed the convenience of conducting the examinations at one single time and the lesser exposure to radiation involved, which has been an increasingly worrying issue in the radiological milieu. In this context, children and pregnant women constitute a subgroup of patients who could undoubtedly benefit from this new technology.

One of the most attractive applications of PET/MRI is in neurology. The brain consists of a complex machine which experiences constant changes from a functional standpoint. Thus, the concurrent correlation between anatomy and activity, besides being convenient, is also extremely relevant for the detection of neurological diseases. It has a high impact on neurodegenerative disorders, such as Alzheimer's disease, epilepsy and brain tumors.

PET/MRI once again offers the complementarity between the molecular information from PET and the anatomical-functional information from MRI for evaluating cardiovascular diseases. It permits a detailed evaluation of myocardial perfusion and contributes to the appropriate therapeutic management.

Accordingly, PET/MRI represents the next generation of the molecular hybrid image, offering great possibility of integration into the clinical routine.

Resumo

Tomografia por emissão de pósitrons/ressonância magnética (PET/RM): atualização e experiência inicial do HC-FMUSP

A nova tecnologia PET/RM é o protótipo de diagnóstico por imagem híbrido e permite combinar dados moleculares obtidos da tomografia PET e informações morfofuncionais derivadas da ressonância magnética. Avanços recentes relativos a aspectos técnicos desse dispositivo, principalmente após o desenvolvimento de fotomultiplicadores de silício compatíveis com RM, permitiram uma melhora do desempenho diagnóstico da PET/RM traduzida em redução da dose e qualidade superior das imagens. Entre diversas aplicações clínicas, a PET/RM ganha espaço inicialmente no campo da oncologia, onde a RM tem papel essencial na avaliação de tumores primários (limitado no caso da PET/TC), incluindo tumores de próstata, reto e ginecológicos. Por outro lado, a avaliação dos pulmões ainda é um enigma, a despeito de novas sequências de RM que estão sendo criadas para tentar resolver essa questão. Outras indicações clínicas da PET/RM são encontradas no âmbito da neurologia, cardiologia e de processos inflamatórios, nos quais a técnica também abre perspectivas para pacientes pediátricos, já que envolve uma exposição baixíssima à radiação. Nossa revisão teve como objetivo destacar as indicações atuais da PET/RM e discutir os desafios e perspectivas da aplicação dessa técnica no Hospital das Clínicas da FMUSP.

Palavras-chave: Tomografia por Emissão de Pósitrons. Tomografia Computadorizada por Raios X. Espectroscopia de Ressonância Magnética. Diagnóstico por Imagem. Revisão.

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Advances in early biomarkers of diabetic nephropathy

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Summary

Diabetic nephropathy is the main cause of chronic kidney disease, and represents the most common and serious complication of diabetes. The exact pathogenesis is complex and not elucidated. Several factors and mechanisms contribute to the development and outcome of diabetic nephropathy. An early diagnosis and intervention may slow down disease progression. A variety of biological markers associated with diabetic nephropathy were found in recent years, which was important for predicting the occurrence and development of the disease. Therefore, this article provides an overview of early biomarkers that are associated with diabetic nephropathy.

Keywords: Diabetes Mellitus. Diabetic Nephropathies. Biomarkers.

Introduction

Diabetes mellitus (DM) is an endocrine and metabolic disease that has serious impact on human health. The morbidity and mortality of DM have risen continually at an alarming rate in recent years, and the population with diabetes mellitus is predicted to be about 439 million worldwide by 2030.[1] The complications of DM include diabetic retinopathy, diabetic cardiovascular diseases and diabetic nephropathy (DN), which is the most common and serious complication of DM. DN has become the leading cause of chronic kidney failure, starting with normoalbuminuria, microalbuminuria, macroalbuminuria and ultimately leading to end stage renal disease (ESRD).[2] For a long time, proteinuria has been considered the gold standard for evaluation and monitoring of renal function. However, renal function declines in about one-third of the patients before the occurrence of proteinuria,[3] which makes it inadequate to detect proteinuria alone to monitor the incidence and progression of DN. Therefore, we need to look for laboratory biomarkers that are earlier than microalbuminuria or those appearing at the same time. This review focuses on the early biomarkers associated with the pathogenesis and pathology of DN and changes in renal function.

Biomarkers associated with DN pathogenesis

A large number of prospective studies confirm that hyperglycemia is the most important risk factor for DN.[4,5] Hyperglycemia promotes mitochondrial electron transport chain to generate excessive reactive oxygen species (ROS) through formation of the advanced glycation end products (AGEs) and activation of the polyol pathway, hexosamine pathway, protein kinase C (PKC) and angiotensin II. Then, the ROS initiate or enhance the oxidative stress and eventually cause the inflammatory response and formation of fibrosis.[6,7] In addition, lipid metabolism abnormality, renin-angiotensin-aldosterone system (RAAS) activation, systemic and glomerular hypertension, insulin signaling impairment, increased growth factors and pro-inflammatory cytokines, and intracellular signaling pathway activation also play a role in the occurrence and progression of DN.[6,8]

Biomarkers of oxidative stress

The occurrence and progression of DN is closely related with oxidative stress. Excessive ROS, which are induced by hyperglycemia, are involved in oxidative stress causing direct oxidation and damage of deoxyribonucleic acid (DNA), proteins and lipids.[8,9]

Biomarkers of DNA injury

8-hydroxy-2'-deoxyguanine (8-OHdG) is a sensitive biomarker of DNA damage to assess oxidative stress in the human body. In 1994, Ha et al.[10] found that the 8-OHdG levels were significantly higher in cortex and nipples of diabetic mice induced by streptozotocin than in control mice, and they decreased after insulin treatment, which

suggested that DN might be associated with oxidative stress and the formation of 8-OHdG. The following study by Hinokio et al.[11] showed that urinary 8-OHdG excretion in patients suffering from type 2 diabetes mellitus complicated by nephropathy was higher than in patients without complications or in healthy control subjects. Moreover, there was a correlation between urinary 8-OHdG level and glycosylated hemoglobin (HbA_{1c}). In this report, 8-OHdG was speculated to be a useful biomarker associated with complications secondary to DM. Zhao et al.[12] measured the serum concentration of 8-OHdG using enzyme-linked immunosorbent assay (ELISA) and drew a similar conclusion. However, Serdar et al. demonstrated that there was no difference in urinary 8-OHdG levels between the groups with and without diabetic nephropathy on liquid chromatography-mass spectrometry, suggesting that 8-OHdG in urine was not a sensitive biomarker regarding albumin to creatinine ratio (UACR) for distinguishing DN patients from DM patients.[13] Different biological fluids and methods might contribute to the lack of consistency in these studies, so that the predictive value of 8-OHdG in the early stages of DN needs further research to be determined.

Biomarkers of protein and lipid injury

Biomarkers associated with protein injury comprise pentosidine, 2,4-dinitrophenylhydrazine (DNPH) and advanced oxidation protein product (AOPP). F2-isoprostaglandin and 4-hydroxy-nonenal (HNE) are related to lipid injury. Calabrese et al. found that both urinary and serum levels of pentosidine, DNPH, F2-isoprostaglandin and HNE of DN patients were higher than those of control subjects.[14] Tabak et al. showed that the level of AOPP in type 2 diabetes mellitus patients with complications such as DN and diabetic retinopathy was significantly higher than in patients without complications.[15] These two studies have confirmed that oxidative stress damage is involved in the development of diabetic nephropathy.

Biomarkers of glutathione antioxidant system and lipid peroxidation

A growing number of studies reported that DM and its complications were closely related to oxidative stress, so we supposed that the biomarkers related to antioxidant defense system and lipid peroxidation (LPO) induced by free radicals may be potential biomarkers of kidney damage in diabetic patients.[8] Glutathione s-transferase (GST), a kind of enzyme involved in cell detoxification, promotes inactivation and excretion of toxins by combining toxic drophobic compounds with glutathione.[8]

Experimental data from a study by Jiang et al. showed that the expression level of GST in diabetic rats induced by streptozotocin was remarkably higher than in control rats, suggesting that hyperglycemia may be the major cause for elevated GST. Eight weeks after treatment with resveratrol, the GST expression decreased and several indicators suggesting the occurrence of DN such as urinary protein excretion, creatinine, cellular apoptosis and renal hypertrophy were all improved, leading researchers to suppose that resveratrol likely played a role in renoprotection by lowering the expression level of GST.[16] In agreement with GST, animal experiments on LPO have yielded the same results.[17,18] In addition, genetic investigation also found that knockout of GST coding genes can lead to decreased GST levels and increased malondialdehyde (MDA) levels, an important biomarker of LPO, demonstrating that GST has an effect against oxidative stress.[19]

Human research was consistent with the experimental studies above. Compared with healthy subjects, increased activity of GST and increased level of MDA were found in type 2 diabetes mellitus patients. These results suggested that oxidative stress was involved in the occurrence of DM and GST was likely to play an important role in antioxidation.[20,21] In the study about GST and DN, Noce et al. reported that GST activity in type 2 diabetes mellitus patients with and without nephropathy were both significantly higher than that of control subjects, appearing to be closely related with the stages of DN and indicating that GST was likely to be a potential biomarker in early stage DN.[22]

Biomarkers of inflammation

Inflammatory response could be activated by biochemical, metabolic or hemodynamic disorders when a large number of white blood cells gather in the kidney. Then, proinflammatory cytokines and a variety of chemokines secreted by leukocytes may guide the latter into the kidney directly. Thus, a new cycle of inflammatory response is induced. The inflammatory cytokines and chemokines involved were hypothesized as potential biomarkers of DN. Liu et al. detected urinary levels of 27 kinds of inflammation-related factors of type 2 diabetes mellitus patients by multiplex-27 bead immunoassay. They found that the levels of proinflammatory cytokines such as interleukin-8 (IL-8), tumor necrosis factor (TNF-α) and chemokines such as monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein-10 (IP-10) in patients with microalbuminuria were all significantly higher than those of patients with normoalbuminuria and

the control subjects. Besides, the levels of MCP-1 and IP-10 were positively correlated with proteinuria and HbA_{1c}, while negatively correlated with the estimated glomerular filtration rate (eGFR).[23] These outcomes suggest that urinary inflammation-related factors may contribute to the diagnosis in early stages of DN.

In addition, some studies have shown that serum interleukin-18 (IL-18) level was elevated in DN patients and associated with HbA_{1c} or UACR, thus being speculated as a potential biomarker of diabetic nephropathy.[24] On the other hand, the value of interleukin-6 (IL-6) in early diagnosis of diabetic nephropathy remains to be further confirmed. A number of studies have found that serum IL-6 levels of patients with normoalbuminuria or microalbuminuria were higher than those of control subjects and showed a positive correlation with UACR.[25-28] However, some other studies have found that serum IL-6 level was elevated in patients with macroalbuminuria alone, and its early diagnosis value was not as good as that of urinary albumin excretion.[24,29]

Some studies demonstrated that an increase in both urinary and serum levels of TNF-α in patients with nephropathy secondary to DM was found compared to those with normoalbuminuria and control subjects. Besides, levels of TNF-α in urine and serum were both significantly associated with urinary albumin excretion. These results revealed that TNF-α might be an early biomarker of kidney damage in diabetic patients.[30,31] Soluble CD40 ligand (sCD40L) is a transmembrane protein of the tumor necrosis factor superfamily and regulates inflammatory response by binding with CD40. A study by El-Asrar et al.[32] showed that serum sCD40L level in type 1 diabetes mellitus patients with microangiopathy such as diabetic nephropathy, retinopathy or neuropathy was significantly higher than that of patients without complications and healthy control subjects, and diabetic patients without any of these complications presented higher sCD40L concentration as compared to healthy subjects. The researchers also found that serum sCD40L was significantly associated with the severity of kidney damage and the level of glycemic control.[33]

In addition to the biomarkers cited above, glycosyl hydrolase family of 18 members, including chitotriosidase (CHIT1) and cartilage glycoprotein 40 (YKL-40), commonly activated by macrophages cells and neutrophils, were also involved in the inflammatory response.[34,35] Several studies showed that both CHIT1 activity and YKL-40 level of type 2 diabetes mellitus patients in all subgroups were higher than that of control subjects. CHIT1 activity and YKL-40 level increased gradually along with the stages of DN according to UACR, which was correlated with activity of CHIT1 and level of YKL-40 even after adjustment for clinical parameters, suggesting that they were both associated with kidney damage of DN patients. However, because of the higher sensitivity and specificity, CHIT1 activity was better in the diagnosis of persistent microalbuminuria compared with serum level of YKL-40.[36,37]

Biomarkers of RAAS activation

Renin-angiotensin-aldosterone system (RAAS) plays an important role in regulating blood pressure by producing aldosterone in human body. Angiotensinogen, produced by liver, was reported in patients with chronic glomerulonephritis in a previous study.[38] The following study found that urinary angiotensinogen excretion of type 2 diabetes mellitus patients with microalbuminuria and macroalbuminuria were both significantly increased compared to control subjects, as well as to normoalbuminuric patients, suggesting that angiotensinogen appeared prior to the establishment of albuminuria. Also, angiotensinogen level shows a strong association with urinary albumin excretion, which is an indicator of the severity of kidney damage in diabetic patients. Angiotensinogen may be a promising biomarker in the early stages of DN due to its high sensitivity and specificity in diagnostic analysis of diabetic nephropathy.[39]

These biomarkers were summarized in Figure 1.

Biomarkers associated with DN pathology

Biomarkers of damage of glomerular filtration membrane

Under normal circumstances, podocyte and foot process, glomerular basement membrane and capillary endothelial cells constitute the glomerular filtration barrier. The damage of this filtration barrier can affect the glomerular filtration function. Markers such as podocytes, basement membrane and endothelial cell damage may have potential to indicate kidney damage in DN patients.

Biomarkers of podocytes injury

Studies have shown that a decline in the number of podocytes and disappearance of foot processes often occur in the early stages of DN due to apoptosis or shedding of podocytes. Therefore, urinary podocytes and their specific protein products may be regarded as potential biomarkers of podocyte injury.[40] Currently, the studies focused on the podocyte-specific protein products because it was difficult to detect urinary podocytes directly. One study by Wang et al.[41] showed that urinary mRNA levels of podocin, synaptopodin and nephrin in DN patients were extremely higher than those found in control subjects

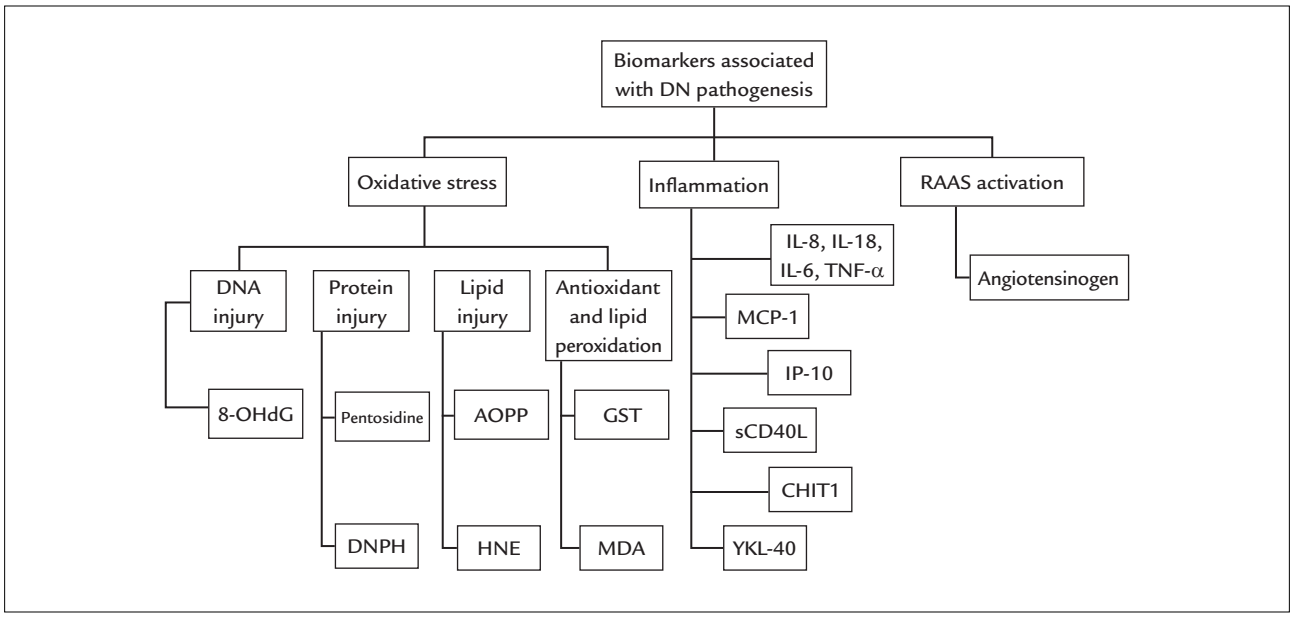

FIGURE 1 Summary of biomarkers associated with DN pathogenesis.

DN: diabetic nephropathy; RAAS: renin-angiotensin-aldosterone system; DNA: deoxyribonucleic acid; IL-8: interleukin-8; IL-18: interleukin-18; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; MCP-1: monocyte chemoattractant protein-1; IP-10: interferon-inducible protein-10; 8-OHdG: 8-dihydro-2'-deoxyguanosine; AOPP: advanced oxidation protein product; GST: glutathione s-transferase; sCD40L: soluble CD40 ligand; CHIT1: chitotriosidase; DNPH: 2,4-dinitrophenylhydrazine; HNE: 4-hydroxy-nonenal; MDA: malondialdehyde; YKL-40: cartilage glycoprotein 40.

by real-time quantitative PCR. These results were also proved by renal biopsy. Also, synaptopodin level was positively correlated with urinary albumin excretion and serum creatinine concentration while negatively correlated with GFR. Patients, however, were not divided into different subgroups according to their average level of urinary protein. The validation of these podocyte-specific protein products in early stages of DN was not confirmed in this study.[41] Further research performed by Hara et al. revealed that urinary synaptopodin level of type 2 diabetes mellitus patients complicated by nephropathy was higher when compared to control subjects, even before the occurrence of proteinuria and associated with the level of urinary albumin and HbA_{1c}, indicating that synaptopodin was a biomarker with high sensitivity to podocyte injury in diabetic patients.[42] Another report by Jim et al. revealed that nephrin level in urine was elevated in all DN patients and 54% of normoalbuminuric subjects. In addition, urinary level of nephrin showed a strong association with UACR so that it might be a useful biomarker for nephropathic patients in preclinical stage.[43]

Biomarkers of basement membrane injury

Type IV collagen is the main component of the glomerular basement membrane and extracellular matrix, and does not pass through glomerular filtration barrier under normal circumstances. Therefore, type IV collagen could be used as a biomarker of basement membrane injury. The study found that urinary type IV collagen levels were higher before microalbuminuria and associated with urinary albumin and serum creatinine, suggesting that urinary type IV collagen may be a promising biomarker for early diagnosis of DN.[44]

Biomarkers of endothelial cells injury

Endothelial cells injury can directly affect the permeability of the glomerular filtration membrane. Generally, von Willebrand factor (vWF) is mostly synthesized by endothelial cells. Plasma vWF levels increase when endothelial cells are stimulated or damaged. Jensen[45] first discovered that plasma levels of vWF are higher in type 1 diabetes mellitus patients, indicating that there is endothelial cell dysfunction in diabetic patients. Subsequently, a number of studies have shown that plasma vWF levels in patients with DN are significantly higher than those in patients without kidney disease and control subjects, indicating that plasma vWF may contribute to the early diagnosis of diabetic nephropathy.[46-48]

Hyperglycemia does aggravate vascular endothelial injury by up-regulating the expression of adhesion molecules by endothelial cells.[49] The study about type 2 diabetes mellitus patients from Malaysia discovered that plasma levels of intercellular adhesion molecule-1 (ICAM-1) are elevated in DN patients.[50]

Vascular endothelial growth factor (VEGF) can affect the filtration of large molecular weight proteins through

glomerular filtration barrier by promoting endothelial cell proliferation and increasing vascular permeability. Researchers have found that plasma and urinary levels of VEGF in DN patients were both elevated. Especially in type 2 diabetes mellitus subjects, urinary VEGF level was higher in normoalbuminuric patients than in control subjects and gradually increased along with the DN stages. These findings suggested that VEGF may be an effective biomarker for early diagnosis in DN patients.[51,52]

Biomarkers of mesangial expansion and fibrosis

Fibrosis is one of the pathological features of diabetic complications caused by extracellular matrix alterations and mesangial expansion. Hyperglycemia up-regulates the expression of transforming growth factor-β1 (TGF-β1), which is considered to be the most crucial cytokine in glomerulosclerosis and tubulointerstitial fibrosis.[53] Data by Xie showed that serum TGF-β1 level of patients with microalbuminuria was significantly higher than that of patients with normoalbuminuria and control subjects. Interestingly, urinary levels of TGF-β1 are already elevated in normoalbuminuria subjects and gradually increase along with DN progression, so that TGF-β1 was considered a sensitive biomarker in the early phase of diabetic nephropathy.[54]

Pigment epithelial-derived factor (PEDF) is a member of the serine protease superfamily and is involved in the formation of extracellular matrix and vascular endothelial growth factor. PEDF levels were found to be decreased in the kidney of diabetic mice, suggesting that it may have a protective effect in diabetic microvascular lesions.[55] Researchers also found that urinary PEDF levels in DN patients are significantly higher than in control patients, indicating that PEDF is probably an effective biomarker of DN.[56]

These biomarkers were summarized in Table 1.

Biomarkers associated with renal function changes

The level of proteinuria in the early stages of DN can tell us whether there is glomerular damage or not and the extent of the damage. Investigation of proteinuria continues to be the gold standard for diagnosis and staging of DN.[57] In addition, albumin, transferrin (TRF), ceruloplasmin (CER) and immunoglobulin G (IgG) in urine can also reflect functional changes in glomerular filtration. Narita et al. found that urinary levels of TRF, CER and IgG in normoalbuminuric patients were significantly higher than those in control subjects and they strongly correlated with each other, indicating that TRF, CER and IgG may be more sensitive makers for changes in filtration function than albuminuria in the early stages of DN.[58]

Biomarkers of renal tubular dysfunction

Tubulointerstitial injury plays an important role in DN development process and even prior to glomerular injury. In addition, about one third of the patients with diabetes mellitus have decreased renal function prior to proteinuria. Therefore, we should pay more attention to the biomarkers of tubulointerstitial injury, which can contribute to the early diagnosis and treatment of DN patients.[59,60]

a1-Microglobulin, retinol-binding protein 4 (RBP4) and other low molecular weight proteins can freely pass through the glomerular filtration membrane and then be reabsorbed in the tubules. These were early biomarkers of tubular injury because of their increase in urine after renal tubular damage. Researchers found that urinary levels of a1-microglobulin and RBP4 in patients with normoalbuminuria were significantly higher than those in control subjects and were both associated with the levels of HbA_{1c}, so that detection of two biomarkers may be helpful for early diagnosis of diabetic nephropathy.[61,62]

Some other biomarkers of tubular injury, such as neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosidase (NAG), kidney injury molecule-1 (KIM-1) and heart-type fatty acid binding protein (H-FABP) applied only to predict acute kidney injury.[63,64] A recent study discovered that urinary levels of NGAL, NAG, KIM-1, H-FABP of patients with normoalbuminuria were significantly higher than those of control subjects and increased gradually along with the DN stages. In addition, they all significantly correlated with urinary albumin levels, indicating that they might be early biomarkers for DN diagnosis.[65]

These biomarkers were summarized in Table 2.

Conclusion

In recent years, there has been an important achievement regarding the finding of associated biomarkers in all aspects of diabetic nephropathy. These research findings contribute greatly to our understanding of disease mechanisms. So far, there is no biomarker that can replace proteinuria. We believe that advances in research methods based on genomics, proteomics and metabolomics will provide much more convenience in future. However, we should also take all the questions into our consideration, such as the fact that there is no universally accepted standard for subject inclusion and staging, not all researches made adjustment for significant parameters and few studies have discussed the effectiveness of multi-biomarker detection.

TABLE 1 Summary of biomarkers associated with DN pathology.

Biomarker	Mechanism	Sample	Method	Study object			Level in DN	Ref
				T2DM	Control 1	Control 2		
Podocin	Podocytes injury	Urine	RT-QPCR	21 DN patients by biopsy	9	—	Increased	[42]
Synaptopodin	Podocytes injury	Urine	RT-QPCR	21 DN patients by biopsy	9	—	Increased	[42]
		Urine	ELISA	71 (39 normo/17 micro/15 macro)	69	—	Increased#	[43]
Nephrin	Podocytes injury	Urine	RT-QPCR	21 DN patients by biopsy	9	—	Increased	[42]
		Urine	ELISA	66 (26 normo/11 micro/29 macro)	10	—	Increased#	[44]
Type IV collagen	Basement membrane injury	Urine	ELISA	698 DM (264 normo/169 micro/181 macro/84 renal failure)	191	—	Increased	[45]
vWF	Endothelial cells injury	Plasma	ELISA	109 (66 normo/26 micro/17 macro)	—	31 nondiabetic	Increased	[47]
		Plasma	ELISA	24 (12 normo/12 micro)	12	—	Increased	[48]
		Serum	ELISA	60 (30 DN/30 without DN)	60	—	Increased	[49]
VEGF	Endothelial cells injury	plasma	ELISA	387 T1DM (188 normo/199 DN)	—	—	Increased	[52]
		Urine	ELISA	107 (37 normo/37 micro/ 33 proteinuria)	47	—	Increased#	[53]
TGF-β1	Mesangial expansion/fibrosis	Serum/ urine	ELISA	54 (20 normo/34 micro)	30	—	Increased	[55]
PEDF	Mesangial expansion/fibrosis	Urine	ELISA	228 (59 normo/130 micro/ 39 macro)	46	—	Increased	[57]

DN: diabetic nephropathy; T2DM: type 2 diabetes mellitus; Ref: reference; RT-QPCR: real-time quantitative polymerase chain reaction; ELISA: enzyme-linked immunesorbent assay; normo: normoalbuminuria; micro: microalbuminuria; macro: macroalbuminuria; DM: diabetes mellitus; vWF: von Willebrand factor; VEGF: vascular endothelial growth factor; T1DM: type 1 diabetes mellitus; TGF-β1: transforming growth factor-β1; PEDF: pigment epithelial-derived factor.
Control 1: healthy subjects; Control 2: not healthy subjects.
#: increased prior to albuminuria.

TABLE 2 Summary of biomarkers associated with renal function changes.

Biomarker	Mechanism	Sample	Method	Study object			Level in DN	Ref
				T2DM	Control 1	Control 2		
TRF/CER/IgG	Glomerular dysfunction	Urine	IRMA	61 (61 normo)	17	—	Increased	[59]
a1-microglobulin	Renal tubular dysfunction	Urine	Latex immunoassay	587 (375 normo/181 micro/31 macro)	—	—	Increased	[62]
RBP	Renal tubular dysfunction	Urine	ELISA	59 T1DM (48 normo/11 micro)	40	—	Increased	[63]
NGAL/NAG/ KIM-1/H-FABP	Renal tubular dysfunction	Urine	ELISA	94 DM (41 normo/41 micro/12 macro)	—	45 nondiabetic	Increased#	[66]

T2DM: type 2 diabetes mellitus; Ref: reference; TRF: transferrin; CER: ceruloplasmin; IgG: immunoglobulin G; IRMA: immunoradiometric assay; normo: normoalbuminuria; micro: microalbuminuria; macro: macroalbuminuria; RBP: retinol-binding protein; ELISA: enzyme-linked immunosorbent assay; T1DM: type 1 diabetes mellitus; NGAL: neutrophil gelatinase-associated lipocalin; NAG: N-acetyl-β-D-glucosidase; KIM-1: kidney injury molecule-1; H-FABP: heart-type fatty acid binding protein; DM: diabetes mellitus.
Control 1: healthy subjects; control 2: not healthy subjects.
#: increased prior to albuminuria.

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