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Análise da relevância do teste de hidrogênio expirado para a abordagem clínica da intolerância à lactose: uma revisão sistemática

Araranguá, SC
2023

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Trabalho de Conclusão de Curso submetido ao curso de graduação em Medicina do Centro de Ciências, Tecnologias e Saúde da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Bacharel em Medicina.

Orientadora: Profa. Cíntia Scherer

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Este Trabalho de Conclusão de Curso foi julgado adequado para obtenção do título de Bacharel e aprovado em sua forma final pelo Curso de Graduação em Medicina.

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Araranguá, 2023.

Aos que ousam sonhar além do que se pode ver.

RESUMO

Contexto: intolerância à lactose é uma patologia prevalente entre cerca de 22% a 34% da população, caracterizada pela disabsorção de lactose associada a sintomas gastrointestinais, como diarreia, náusea, distensão e dor abdominal. Para fins diagnósticos, portanto, necessita-se de métodos que evidenciem simultaneamente sintomatologia e disabsorção. Abordagens disponíveis são: atividade da lactase em biópsia duodenal, galactose urinária, gaxilose sérica, genotipagem, teste oral de tolerância à lactose e teste de hidrogênio expirado. Este, empregado de modo não invasivo, mensura os níveis de gás hidrogênio exalado, oriundo da fermentação da lactose pelo microbioma colônico. **Objetivos:** analisar o emprego do teste de hidrogênio expirado para o diagnóstico de intolerância à lactose. **Métodos:** consultaram-se os trabalhos disponíveis até 31 de outubro de 2022 nas bases Biblioteca Virtual em Saúde, COCHRANE Library, EMBASE, PubMed, SciELO e Scopus. Aplicados os critérios de elegibilidade, os autores, independentemente, avaliaram manualmente as variáveis e suas respectivas medidas de tendência central em uma planilha no Microsoft Excel®. **Resultados**: a partir de 50 artigos selecionados, sendo 68% de origem europeia e 46% da década de 2010, evidenciou-se elevada heterogeneidade quanto ao emprego do teste de hidrogênio expirado, com 33 protocolos distintos. Com isso, os trabalhos não determinaram precisamente seus potenciais diagnósticos, vantagens, limitações e eventos adversos de modo homogêneo, inviabilizando análises seguras. **Conclusão**: constatou-se benefícios que defendem o uso do teste de hidrogênio expirado no diagnóstico de intolerância à lactose. No entanto, a falta de um protocolo norteador e as múltiplas abordagens põem em risco essas vantagens.

Palavras-chave: Intolerância à lactose. Teste de hidrogênio expirado. Teste de tolerância à lactose. Técnicas e procedimentos diagnósticos. Técnicas de diagnóstico do sistema digestório.

ABSTRACT

Background: lactose intolerance is a pathology prevalent among about 22% to 34% of the population, characterized by lactose malabsorption associated with gastrointestinal symptoms such as diarrhea, nausea, distension and abdominal pain. For diagnostic purposes, therefore, are needed methods that simultaneously show symptoms and malabsorption. Approaches available are: lactase activity in duodenal biopsy, urinary galactose, serum gaxylosis, genotyping, lactose tolerance test and hydrogen breath test. This, used in a non-invasive way, measures the levels of exhaled hydrogen gas, derived from the fermentation of lactose by the colonic microbiome. **Objective:** to analyze the use of the hydrogen breath test for the diagnosis of lactose intolerance. **Methods:** the papers available up to October 31, 2022 were consulted in the Virtual Health Library, COCHRANE Library, EMBASE, PubMed, SciELO and Scopus databases. After applying the eligibility criteria, the authors, independently, manually evaluated the variables and their respective measures of central tendency in a spreadsheet in Microsoft Excel®. **Results:** from 50 selected articles, 68% from Europe and 46% from the 2010s, a high heterogeneity was evidenced regarding the use of the hydrogen breath test, with 33 different protocols. As a result, the studies did not precisely determine their potential diagnoses, advantages, limitations and adverse events in a homogeneous way, making safe analyzes unfeasible. **Conclusion:** benefits were found that support the use of the breath hydrogen test in the diagnosis of lactose intolerance. However, the lack of a guiding protocol and the multiple approaches put these advantages at risk.

Keywords: Lactose intolerance. Hydrogen breath test. Lactose tolerance test. Diagnostic techniques and procedures. Diagnostic techniques, digestive system.

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Analysis of hydrogen breath test relevance on clinical approach of lactose intolerance: a systematic review

Análise da relevância do teste de hidrogênio expirado na abordagem clínica da intolerância à lactose: uma revisão sistemática

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ABSTRACT

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Contexto: intolerância à lactose é uma patologia prevalente entre cerca de 22% a 34% da população, caracterizada pela disabsorção de lactose associada a sintomas gastrointestinais, como diarreia, náusea, distensão e dor abdominal. Para fins diagnósticos, portanto, necessita-se de métodos que evidenciem simultaneamente sintomatologia e disabsorção. Abordagens disponíveis são: atividade da lactase em biópsia duodenal, galactose urinária, gaxilose sérica, genotipagem, teste oral de tolerância à lactose e teste de hidrogênio expirado. Este, empregado de modo não invasivo, mensura os níveis de gás hidrogênio exalado, oriundo da fermentação da lactose pelo microbioma colônico. **Objetivos:** analisar o emprego do teste de hidrogênio expirado para o diagnóstico de intolerância à lactose. **Métodos:** consultaram-se os trabalhos disponíveis até 31 de outubro de 2022 nas bases Biblioteca Virtual em Saúde, COCHRANE Library, EMBASE, PubMed, SciELO e Scopus. Aplicados os critérios de elegibilidade, os autores, independentemente, avaliaram manualmente as variáveis e suas respectivas medidas de tendência central em uma planilha no Microsoft Excel®. **Resultados**: a partir de 50 artigos selecionados, sendo 68% de origem europeia e 46% da década de 2010, evidenciou-se elevada heterogeneidade quanto ao emprego do teste de hidrogênio expirado, com 33 protocolos distintos. Com isso, os trabalhos não determinaram precisamente seus potenciais diagnósticos, vantagens, limitações e eventos adversos de modo homogêneo, inviabilizando análises seguras. **Conclusão**: constatou-se benefícios que defendem o uso do teste de hidrogênio expirado no diagnóstico de intolerância à lactose. No entanto, a falta de um protocolo norteador e as múltiplas abordagens põem em risco essas vantagens.

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1 INTRODUCTION

Lactose intolerance (LI) is a condition characterized by the association between intestinal malabsorption of lactose and gastrointestinal symptoms following the ingestion of products containing this disaccharide[1]. Globally, the prevalence of lactose malabsorption is approximately 68%, varying according to ethnic and regional factors. However, since LI depends not only on the intestinal deficiency in absorbing this sugar but also on the simultaneous presence of abdominal symptoms, its prevalence remains uncertain, with one-third to half of those with malabsorption also being intolerant[2].

Regarding the etiopathogenesis of this disorder, its main cause is linked to a deficiency of the enzyme lactase, responsible for hydrolyzing lactose into glucose and galactose. This deficiency can be congenital, a rare form that causes symptoms in infants; primary, the most common form, resulting from genetic polymorphisms associated with lactase non-persistence throughout life; and secondary, caused by surgical, infectious, and inflammatory processes, often being transient[3,4]. In summary, in the absence of this enzyme, lactose reaches the large intestine undigested and comes into contact with the bacterial flora of the colonic mucosa, which ferments this disaccharide, producing short-chain fatty acids and gases such as methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2). The accumulation of these substances leads to symptoms such as diarrhea, abdominal pain and bloating, flatulence, nausea, and constipation[1].

In order to diagnose lactose intolerance, it is necessary to not only perform investigative tests for the malabsorption of this sugar but also conduct a concomitant symptom-related interview[3,4]. There are several approaches available for detecting this enzyme deficiency, including genetic testing, serum gaxilose or galactose in urine testing, evaluation of lactase activity in the duodenum, oral lactose tolerance test, and hydrogen breath test (HBT)[1].

Genotyping aims to assess the genetic polymorphism associated with lactase non-persistence (primary deficiency), but its use is uncommon in clinical practice and is mainly restricted to epidemiological studies[5]. Serum gaxilose and galactose in urine testing involve the detection of monosaccharides in urine or serum resulting from the hydrolysis of a synthetic lactose analog. As for the evaluation of duodenal lactase, it entails the analysis of duodenal biopsy to identify lactase activity. This

method was previously considered the gold standard for detecting lactose malabsorption; however, its highly invasive nature and difficulty in assessing the irregular enzymatic distribution throughout the intestinal mucosa have raised questions about its accuracy. The oral lactose tolerance test, on the other hand, is widely used clinically and involves measuring serum glucose levels after the ingestion of a lactose dose. However, this test requires a minimum of three venous blood samples taken within a short period of time, and there are limitations regarding changes in body metabolism[6].

Finally, the HBT is based on the production of hydrogen by the bacterial flora of the colonic mucosa[7]. Through the fermentation of lactose in this organ, hydrogen gas is produced and diffuses through the mucosa into the adjacent bloodstream, reaching the lungs, where it is again diffused into the airways and exhaled[4,8]. This test utilizes an expired air analyzer based on gas chromatography. As the exhaled air passes through the device, the hydrogen travels through a column system and is gradually separated from the rest of the exhaled sample. At the end of the column, it is detected by a sensor that transmits the numerical information to the display of the device. In this test, after administering 20 to 25 g of lactose, hydrogen levels are measured every 30 minutes for three hours, following the most up-to-date guidelines in gastroenterology. Therefore, the detection of hydrogen levels exceeding the basal rate by 20 ppm corresponds to a diagnosis of intestinal malabsorption, which, if accompanied by characteristic symptoms, indicates lactose intolerance[3,4,9].

Based on the available literature up until October 2022, it is worth questioning the relevance of the hydrogen breath test compared to other diagnostic methods for the clinical management of lactose intolerance. The HBT has significant potential for use in clinical practice, as it allows for the simultaneous measurement of intestinal lactose malabsorption levels and the recording of gastrointestinal symptoms, while being practical and non-invasive.

2 METHODS

2.1 RESEARCH DELINEATION

This study, of bibliographic nature, registered in PROSPERO with number CRD42023431181, aimed to conduct a systematic literature review that focused on

the relevance of the hydrogen breath test compared to other diagnostic methods for the clinical approach to lactose intolerance, based on the literature available until October 2022. As recommended by Galvão and Pereira (2014), the research problem is correlated with the acronym PICOS (derived from English, corresponding to Population, Intervention, Comparison, Outcome, and Study type), as demonstrated in Figure 1[10].

2.2 DATABASE

The electronic databases selected for the bibliographic research were: Biblioteca Virtual em Saúde, COCHRANE Library, EMBASE, PubMed, SciELO, and Scopus. In these databases, the searches were conducted from August 25, 2022, to October 31, 2022, retrieving works in their final and complete version published from the inception of these databases until October 31, 2022.

2.3 SEARCH STRATEGY

During the development of the bibliographic research, the construction of the search strategy incorporated terms for lactose intolerance, hydrogen breath test, and other applicable diagnostic methods. Therefore, standardized keywords were adopted, whenever possible, according to the Medical Subject Headings (MeSH) and/or Descritores em Ciências da Saúde (DeCS).

Thus, a common search strategy was applied across the databases, as much as possible, following the template: ("Lactose Intolerance" OR "Alactasia" OR "Dairy Product Intolerance" OR "Hypolactasia" OR "Intolerance, Lactose" OR "Lactose Malabsorption" OR "Malabsorption, Lactose" OR "Milk Sugar Intolerance" OR "Lactose sensitivity" OR "Glucose-galactose malabsorption" OR "Lactase deficiency" OR "Lactase persistence" OR "Lactase non-persistence" OR "Carbohydrate Malabsorption" OR "Cow Milk Intolerance") AND ("Hydrogen breath test" OR "Breath hydrogen test" OR "HBT" OR "Breath Tests" OR "Breath Test" OR "Test, Breath" OR "Tests, Breath" OR "Breathalyzer Tests" OR "Breathalyzer Test" OR "Test, Breathalyzer" OR "Tests, Breathalyzer") AND ("Lactose Tolerance Test" OR "Lactose Tolerance Tests" OR "Test, Lactose Tolerance" OR "Tests, Lactose Tolerance" OR "Tolerance Test, Lactose" OR "Tolerance Tests, Lactose" OR "Genetic Testing" OR

"Testing, Genetic" OR "Testing, Genetic Predisposition" OR "Predisposition Testing, Genetic" OR "Predictive Testing, Genetic" OR "Genetic Predictive Testing" OR "Testing, Genetic Predictive" OR "Predictive Genetic Testing" OR "Genetic Testing, Predictive" OR "Testing, Predictive Genetic" OR "Genetic Predisposition Testing" OR "Genetic Screening" OR "Genetic Screenings" OR "Screening, Genetic" OR "Screenings, Genetic" OR "Genetic test" OR "Polymorphism, Genetic" OR "LCT gene" OR "Duodenal lactase activity" OR "Serum gaxilose" OR "Urine galactose test").

Regarding the search limits, the selected studies were those published in Portuguese, English, and Spanish, in their final version and fully available in the databases used.

2.4 STUDY SELECTION

The main research was conducted independently by the two researchers in separate contexts, and the volume of tracked papers was exported into spreadsheets for management and manual analysis in Microsoft Excel®. In this process, duplicates were removed, as well as those that did not meet the criteria explained in this methodology.

Once the group of eligible articles was established, the eligibility criteria were applied separately by the two authors in different situations. The evaluation of the content of the articles was conducted through a hierarchical reading system. The types of readings employed in this study, in order of application, were: (1) exploratory reading based on the title and abstract of the articles; (2) selective reading aimed at assessing eligibility criteria; (3) analytical reading for data collection; (4) interpretative reading, correlating the studies with each other. If an exclusion criterion was identified at a particular reading level, the subsequent reading mode was not performed, and the study was eliminated[13]. Upon completion of this methodological phase, the group of included articles was obtained.

2.4.1 Eligibility Criteria

2.4.1.1 Inclusion Criteria

Regarding the type of study, the following were included: intervention studies, both randomized and non-randomized, as well as observational studies of any kind. Therefore, original studies published in their final version and fully available in the consulted databases were selected, whether through open access or institutional access. Furthermore, papers in Portuguese, English, and Spanish languages were considered.

In terms of research design, original articles with qualitative or quantitative data on lactose intolerance and its diagnostic methods were considered. Additionally, studies that used lactose alone as a substrate for the hydrogen breath test were included (tests with substrates such as glucose or lactulose were excluded).

Finally, it should be noted that articles with weak methodology were also included. Weak methodology is defined here as studies lacking data on the number of participants, insufficient description of the methodology, or populations or samples with an increased risk of bias. The aim was to evaluate the methodological quality present in the current literature.

2.4.1.2 Exclusion Criteria

Regarding the study characteristics, the following were excluded: any type of review articles and studies that were not published in their final version or were not available in their full version. Studies in languages other than those mentioned in the Inclusion Criteria were also disregarded.

In terms of the content of the articles, the following were not included: studies lacking substantial data on lactose intolerance or those that did not address at least one of the following topics: definition, etiology, epidemiology, pathophysiology, clinical presentations, diagnostic approach. Similarly, articles with insufficient qualitative or quantitative data on lactose intolerance diagnostic methods were removed, requiring that at least one diagnostic test be included in the selected studies.

2.5 QUALITATIVE EVALUATION AND RISK OF BIAS IN THE STUDIES

In order to construct a high-quality bibliographic research, the researchers will follow the recommendations of Ma et al. (2020) regarding the assessment of quality

and risk of bias in the included studies. As mentioned by the authors, there are several tools available currently for assessing the risk of bias, with the Joanna Briggs Institute having the largest portfolio. Therefore, in this study, depending on the research design of each included study, the Critical Appraisal Tools from the Joanna Briggs Institute will be employed, which are available on the institute's website. This assessment of the selected papers will be conducted on the entirety of the texts, rather than solely considering the results[11].

2.6 DATA COLLECTION

Once the group of included articles was established, the researchers manually conducted data extraction into a spreadsheet in Microsoft Excel®. This process was done independently, and any inconsistencies were addressed in consensus meetings. The mentioned spreadsheet was composed according to the categories of variables described in Figure 2.

2.7 DATA ANALYSIS

The included studies underwent the hierarchical reading system as presented in the Study Selection section, and data were extracted based on the previously discussed variables. After completing the data collection, the authors proceeded with exploratory analysis based on measures of central tendency, as needed. Extensive epidemiological and statistical analyses were not considered in this article.

Therefore, the aim was to analyze the methodological quality of the included articles, stating the attributed risk of bias. This fact was considered in the discussion of the overall results and any inconsistencies, with priority given to evidence from journals with a higher impact factor.

3 RESULTS

3.1 CONTEXTUAL VARIABLES

In this analysis, it was observed that 68% of the publications originated from Europe, 16% from the Americas, 14% from Asia, and 2% from Oceania. The

countries with the most studies included Italy (22%), Austria (10%), Spain (8%), India (8%), Poland (8%), Brazil (6%), Chile (6%), and the United Kingdom (6%). Regarding the publication periods, there was greater activity between the years 2011 and 2020 (46%), followed by 2001-2010 (36%). The most productive year was 2008 (10%).

In terms of the journals of the studies, there was a great heterogeneity, with the highest frequency of publications in Nutrients (8%), Archives of Disease in Childhood (6%), and Digestive and Liver Disease (6%). Regarding the research designs, there was a predominance of cross-sectional studies (94%), with the remaining being cohort studies (6%). Randomization was not performed in 90% of the articles, and blinding was adopted in only 12% of the studies.

Regarding the demographic profile of the samples, a total of 9,826 participants were considered, of which 34% were female, 22% were male, and 44% were not reported. In terms of age groups, 45% were adults, 15% were children and adolescents, 9% were the general population, and 32% were not specified.

3.2 DIAGNOSTIC VARIABLES OF LACTOSE INTOLERANCE

Regarding the diagnostic method of the Hydrogen Breath Test (HBT), the criterion of an increase of 20 ppm or more above the baseline level of expired H_2 measured in two consecutive readings was found to be the most prominent. Only one article deviated from this apparent consensus, considering an increase of 25 ppm for diagnosis. The other reported methods included detection of genetic polymorphisms (13910CC, 13910CT, 22018GG, 22018GA), slight colorimetric changes in the duodenal biopsy test, elevations of less than 1.0 to 1.1 mmol/L (or 20 mg/dL) in serum glucose concentration, and urinary galactose/creatinine ratio less than 0.10 mg/mg.

Regarding the volumes of tests performed, the HBT was used in 100% of the articles, followed by genotyping (64%), lactose tolerance test (32%), intestinal biopsy (10%), urinary galactose (2%), urinary galactose (2%), and serum galactose (2%).

In terms of the diagnostic values of the tests, the HBT showed sensitivity (S) values ranging from 37.00% to 100.00%, specificity (E) values ranging from 62.00% to 100.00%, positive predictive value (PPV) values ranging from 67.60% to 100.00%, and negative predictive value (NPV) values ranging from 41.70% to 100.00%. For genotyping, the values were S 75.00% to 100.00%, E 46.00% to 99.00%, PPV

60.00% to 97.00%, and NPV 81.20% to 100.00%. As for the lactose tolerance test, the values were S 64.30% to 96.60%, E 58.80% to 88.60%, PPV 52.90% to 96.60%, and NPV 27.80% to 90.90%.

3.3 VARIABLES OF THE HYDROGEN BREATH TEST

Regarding the equipment used in the HBT, there is a predominance of the Breath Tracker Microlyzer® and Gastrolyzer Breath H_2 Monitor® devices, originating from the United States and the United Kingdom, respectively, which accounted for 74% of the devices used in the studies. Thus, despite being responsible for almost 80% of the equipment in the study, these two countries accounted for only 8% of the research in the field, indicating a predominance in the production of technologies in this regard.

Regarding the protocols employed, there was a wide variation in the doses of lactose used, with the following highlights: 50 g (34%), 25 g (30%), 2 g/kg of body weight (16%), 1 g/kg of body weight (10%) - it should be noted that the last two set the maximum dose at 50 g and 25 g, respectively.

Concomitant with the variability in lactose dosage, there was a large fluctuation in the testing times, both in the interval between samples and in the total duration of the HBT. Out of the total of 50 selected studies, 33 different testing methodologies were observed, as presented in Table 1. The most commonly used protocols in terms of time were: (a) 7 samples every 15 minutes, totaling 3 hours of testing (28%), and (b) 9 samples every 30 minutes, totaling 4 hours (24%). Protocols with less than 2 hours or more than 4 hours were correlated with lower sensitivity, specificity, and positive and negative predictive values.

Regarding the reported adverse symptoms during the test in patients suspected or positive for lactose intolerance, the most frequent ones were diarrhea (84%), abdominal distension (72%), abdominal pain (66%), flatulence (64%), nausea (40%), bloating (26%), abdominal colic (18%), vomiting (18%), constipation (12%), and headache (8%). Other less frequent symptoms not related to the gastrointestinal tract were: skin changes (6%), bronchial asthma (4%), palpitations (4%), polyuria (4%), and dizziness (4%).

Finally, regarding patient preparation prior to the test, the following measures were most frequently reported: a low-fermentation diet 1 day before the test (46%),

fasting for 10 to 12 hours (44%), avoiding smoking and/or physical exertion for 30 minutes to 2 hours before the test (32%), fasting for 8 hours (26%), oral hygiene before the test (26%), lactose-free diet for 1 to 2 days before the test (14%), fasting for at least 3 to 6 hours for children under 6 years old (6%). In addition, the use of antibiotic therapy, medications that alter gastrointestinal motility or microbiota, gastrointestinal procedures, and other factors occurring 1 to 4 weeks before the tests were impediments to performing the HBT in 20% of the articles.

4 DISCUSSION

When discussing lactose intolerance (LI), there is a consensus regarding the association between intestinal lactose malabsorption and gastrointestinal symptoms after the ingestion of dairy products and derivatives[3,4]. Therefore, when analyzing the use of the hydrogen breath test (HBT) for the diagnosis of LI, this study summarizes and compares diagnostic methodologies adopted in the available literature.

It is pertinent to note that this research mainly focused on indirect methods of detecting LI. These methods involve the analysis of serum, urine, or gas factors that somehow indicate a malabsorption of the disaccharide in question. The mentioned methods included the hydrogen breath test, genotyping, oral lactose tolerance test, serum gaxilose, and urinary galactose. Thus, only one of the tests included in the study consists of the objective and direct measurement of lactase deficiency: the analysis of duodenal mucosa. Therefore, when correlating such tests, possible correlations were analyzed among the research results regarding the profile of the articles, the diagnostic methods used, the methodology employed in the use of HBT, and the clinical manifestations presented during the tests.

Firstly, when evaluating the profile of the research in terms of population characteristics, this study involved 9826 patients. Regarding age, there is a highlight for the adult age group, which corresponds to 45% of the total studied, compared to 15% for children and adolescents, and 32% for the population with unspecified age range. This difference makes it clear that there is a preference in the literature for the adult population. Based on the study of the articles, it can be inferred that research involving HBT in children and adolescents presents certain limitations, especially among the younger ones. This can be observed in researches that highlight

difficulties and changes in evaluation techniques mainly among infants and preschoolers, who are uncooperative during the test[22,23,25,27]. As for gender evaluation, the studies show a predominance of unspecified gender (44%), followed by women (34%) and men (22%), with no plausible reasons found for this difference.

Regarding the spatial distribution spectrum in research in the field, there is a predominance of European countries, as the continent accounted for 68% of the analyzed research in this study, followed by the Americas (16%) and Asia (14%). Consequently, there is a deficit in the literature as the concentration of research in one region impoverishes the epidemiological considerations about the pathology. Among the countries with the highest volume of research are Italy (22%), Austria (10%), Spain, Poland, and India (8%). It is worth noting India in particular, as it deviates from the European axis, and the articles highlight different ethnic compositions in the country as a preponderant factor for the epidemiological differences in lactose intolerance[14,36,48,62].

Additionally, when analyzing the temporal distribution of research since the beginning of the databases, there is a significant growth in interest in the field from the 21st century onwards, with 36% of the articles produced from 2001-2010 and 46% from 2011-2020. Furthermore, when evaluating the journals in which the included works were published, the journals Nutrients stand out with 8% of the publications, followed by the journals Archives of Disease in Childhood and Digestive and Liver Disease, both with 6% of the total publications.

Regarding the types of studies conducted, it is observed that 94% of them are cross-sectional studies, with only 10% of the total being randomized and 12% blinded. However, this demonstrates a significant weakness in the literature regarding the diagnosis of LI since there is stronger evidence from blinded and randomized clinical trials[12].

In terms of the research profile regarding the diagnostic evaluation for LI, a total of 8,397 hydrogen breath tests and 7,661 other tests were conducted. In addition to the HBT, which was present in 100% of the articles (as it is an item of analysis), there was a highlight on genotyping (64% of the articles and 30.19% of the total tests) and the oral lactose tolerance test (32% of the articles and 15.09% of the total tests), while intestinal biopsy was present in 10% of the articles (4.72% of the total tests), and the tests for serum gaxilose and urinary galactose together accounted for 6% of the articles and 2.82% of the total. However, a possible

limitation of the study is that the profile of tests used may not represent reality since the inclusion criteria for the review only involved articles with HBT, thereby excluding other works that addressed the other tests without their simultaneous performance.

When analyzing these relationships, it is noticeable that genetic evaluation stands out compared to HBT. According to the studies, this method has the advantage of being independent of adequate patient preparation, non-invasive, and could be useful in cases where the evaluation by hydrogen breath test is challenging, such as in uncooperative children[22,23,34,36]. However, based on the available literature and as predicted by the authors, it can be inferred that although useful for detecting primary forms of lactose malabsorption and its clinical manifestations, this method has limitations in evaluating secondary forms of the disease. This can be observed as the identification of the polymorphisms in question is independent of the coexistence of surgical, infectious, or inflammatory factors that may also be associated with lactose malabsorption[23,29,35,37].

As for the oral lactose tolerance test, there seems to be a possible decline in its academic approach to LI. While it is commonly used in clinical practice, according to the authors' empirical evaluation, there is a tendency to decrease experimental use of this test. Literature elements supporting this divergence may be associated with fluctuations in serum glucose levels due to endocrine factors (such as diabetes mellitus) or gastrointestinal factors (such as variations in peristaltic rhythm and absorptive and distributive potentials). In addition, other relevant factors to consider as limitations are its invasive nature, patient-dependent preparation requirements, and the supraphysiological doses used to perform the test, which not only increase patient discomfort but also the rate of false positives. It is worth noting that the commonly employed dosage of 50 grams of lactose is equivalent to approximately 1 liter of milk, a volume that is rarely consumed in daily dietary references[20,23,36,42,51].

Regarding intestinal biopsy for the detection of lactase activity, it was mentioned in the literature as the gold standard for detecting intestinal lactose malabsorption. However, as expected by the authors, studies show a tendency to replace it with HBT as the diagnostic standard, as intestinal evaluation is limited to samples from the initial portion of the duodenum, while lactase activity is more relevant in the jejunum and ileum. Furthermore, it is also mentioned that intestinal biopsy is highly invasive and not clinically practical, as it can only be performed endoscopically[20,34,53].

Furthermore, it is important to consider the advent of a new indirect technique: the detection of gaxilose in serum or urine. Despite being recent, one study showed that it had sensitivity, specificity, and positive and negative predictive values above 90%. However, there are few studies and limited clinical use of this technique, requiring more research for evaluation and accuracy[53].

Therefore, when analyzing the tests together and considering the relevance of HBT, it is evident that HBT has advantages such as simplicity of performance, easy reproducibility, non-invasiveness, and good accuracy in determining the phenotype for lactose intolerance[17,22,23,32,38]. As for its limitations, HBT is unable to differentiate the degree of hypolactasia, may produce uncomfortable symptoms, and its sensitivity can be affected by factors such as small intestinal bacterial overgrowth[17,26,34,53].

The authors highlight a significant distinction in the methodological approach of studies using HBT. Through the analysis, it can be correlated that higher sensitivity and specificity are associated with protocols using 25 or 50 grams of lactose in doses and durations longer than 3 hours. In these protocols, the values approach 90% in the indicators, which aligns with the authors' empirical observations in their clinical practice. Thus, the wide range reported in the results reflects the significant heterogeneity of protocols used in the literature[14,30,36,53,54,61].

Based on these data, when analyzing the diagnostic criteria for lactose intolerance, most articles consider HBT as the basis for confirming lactose malabsorption. Therefore, it is necessary to have an increase in expired hydrogen levels above 20 ppm from the baseline value in two consecutive readings for the test to be considered positive[14]. However, when considering the oral lactose tolerance test, positivity is determined by a serum glucose increase of less than 1.0 to 1.1 mmol/L (or 20 mg/dL). In the case of genetic testing, only the identification of the 13910CT polymorphism is sufficient. Only one article uses a cutoff point of 25 ppm increase above baseline in HBT, deviating from the consensus of the others[44]. Additionally, other articles present minority criteria with low support in the consensus. However, there is a substantial flaw in the literature, as less than 20% of the studies consider lactose intolerance as the association between lactose malabsorption and the presence of symptoms after its ingestion, which is a basic concept in defining the syndrome[17,18,19,21,26,27,42,46,60]. The authors relate this issue to the poor distinction between the concepts of lactose malabsorption and lactose intolerance, with the former being a pathophysiological mechanism and the latter being a clinical entity.

Regarding the methodology applied to perform the hydrogen breath tests, the equipment used, dose, test duration, sampling interval, and patient preparation were evaluated.

In terms of the doses applied, it is worth mentioning the recommendations for hydrogen breath tests, which advocate the use of 20 to 25 grams of lactose for the tests, approximating physiological and dietary conditions[3,4]. Therefore, it was expected to find a predominance of studies using doses within this range. However, in over half of the articles, doses ranging from 40 to 50 grams of lactose were used, which are considered supraphysiological and poorly tolerable even in individuals without the pathology. It is important to note, though, that there is a trend towards an increased adoption of lactose amounts as recommended, so recent studies predominantly utilize 20 to 25 grams of lactose as the required substrate for the test.

Regarding the test duration and sampling interval, guidelines recommend a total test duration of 3 to 5 hours with a sampling interval of 15 to 30 minutes[3,4]. As expected, the majority of studies did indeed use these values, with 80% of the research adopting a total test duration of 3 to 4 hours. Only 12% conducted the tests for less than 2 hours, 6% for more than 5 hours, and the remaining did not specify the total time. However, according to the consensus and guidelines, total test durations below or above the recommended range carry a higher risk of decreased test sensitivity, which can affect the results. In terms of the sampling interval, it is observed that 84% of the research studies followed the recommended intervals.

When it comes to the reported symptoms during the tests, the most common ones are abdominal distension and pain, diarrhea, flatulence, and nausea, with minor systemic symptoms also reported. However, there is a weakness in the studies as the majority of them do not specify the quantities and characteristics of the symptoms reported by patients during the tests. Therefore, it is not possible to confidently compare the impacts between the 25 g, 50 g, and less frequent dosages in HBT. Among the few studies that provided more concrete data, the testing was predominantly performed in adults with a positive diagnosis of lactose intolerance. In four articles using 25g of lactose as the substrate for HBT (sample size of 1,758 patients), abdominal distension was reported in 55.30% to 83.30% of the patients, nausea in 25.00%, flatulence in 1.25% to 79.00%, diarrhea in 10.00% to 20.00%, and abdominal pain in 1.10% to 58.00%(15,33,41,59). In another three articles using 50g of lactose as the substrate for HBT (sample size of 406 patients), abdominal

distension and nausea were reported in 25.00% of the patients, flatulence in 22.80% to 72.00%, diarrhea in 19.20% to 44.00%, and abdominal pain in 41.00% to 45.00%(34,60,63). Thus, there appears to be no significant difference in the clinical presentation between the use of 25 g or 50 g of lactose. However, it is necessary to emphasize that there is a considerable discrepancy between each sample, and the number of articles represents a small fraction of the total, making it impossible to establish with certainty whether both dosages have the same efficacy, as well as their actual percentages of adverse symptoms. Furthermore, out of the total of 232 symptom mentions in the selected studies, the age group with the fewest symptoms is children and adolescents, with 24 reported symptoms (10.34%). However, it should be noted that there is a risk of bias due to the smaller number of studies on pediatric populations regarding HBT since out of the 50 studies selected in this review, only 8 had exclusively children and adolescents as the sample.

5 CONCLUSION

This article aimed to analyze the use of the hydrogen breath test for the detection of lactose intolerance. Thus, in line with the fact that this technology has gained popularity, an expansion of research in the field has been observed over time.

Furthermore, it was possible to conclude that the available literature indeed considers the hydrogen breath test superior to other diagnostic approaches, mainly due to its advantages. Although it has some limitations, these limitations are of little relevance to clinical practice, leading to the frequent consideration of this test as the new gold standard.

However, it is important to highlight a certain fragility in the literature regarding the standardization of the hydrogen breath test, with some discrepancies among studies concerning the doses used and relevant diagnostic criteria for the characterization of lactose intolerance.

Therefore, although it was possible to establish a foundation on the subject, more studies in the field need to be conducted in order to consolidate knowledge about the best execution protocols of the hydrogen breath test, its diagnostic values, adverse effects of the doses used, and the availability of clinical application. Such studies will be important to validate a concise diagnostic approach that leads to appropriate and efficient treatments.

AUTHOR'S CONTRIBUTION

The Contributor Role Taxonomy (CRediT) was employed in this review article to standardize and validate the roles of each author. This instrument allowed for the attribution of specific contributions to each author involved in the study[64].

Mateus H. R. de Almeida: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, project administration.

Victor L. R. da Silva: conceptualization, methodology, validation, investigation, data curation, writing – original draft, writing – review and editing, visualization, project administration.

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FIGURES

Component	Acronym	Description
Population	P	No population exclusion criteria will be applied, in addition to those already applied in the included studies
Intervention	I	Hydrogen breath test
Comparison	C	Other diagnostic tests for lactose malabsorption - genotyping, intestinal biopsies, lactose tolerance test, urinary galactose excretion
Outcome	O	Identification of the potentialities and limitations of the hydrogen breath test (HBT) compared to other tests for the diagnosis of lactose intolerance, enabling the construction of a profile of situations in which HBT is most indicated
Study type	S	Original papers published in their final version until October 2022, in indexed journals, fully available in the selected bibliographic databases

HBT: *hydrogen breath test*.

Figure 1. Application of the PICOS acronym in this research (Source: the authors).

Categories	Variables
Contextual variables	Title of the article, authors, country of affiliation of the first author, year of publication, journal, impact factor, source database, objectives of the article, research design.
Qualitative variables	Main results, study advantages, study limitations, risk of bias.
Variables related to lactose intolerance	Definition of the disease, etiology, pathophysiology, clinical presentations, diagnostic criteria, employed diagnostic tests.
Variables related to HBT	Equipment, standard dose of lactose, number of collections, expiration time, interval between collections, total examination time, patient preparation quality, reported symptoms during the test, qualification of reported symptoms, number of participants, advantages, limitations, sensitivity, specificity, positive predictive value, negative predictive value, sociodemographic profile of patients.
Variables related to other diagnostic tests for lactose intolerance	Equipment, standard substrate dose, number of collections, interval between collections, total examination time, quality of patient preparation, reported symptoms during the test, qualification of reported symptoms, number of participants, advantages, limitations, sensitivity, specificity, positive predictive value, negative predictive value, sociodemographic profile of patients.

HBT: *hydrogen breath test*.

Figure 2. Variables adopted in this research (Source: the authors).

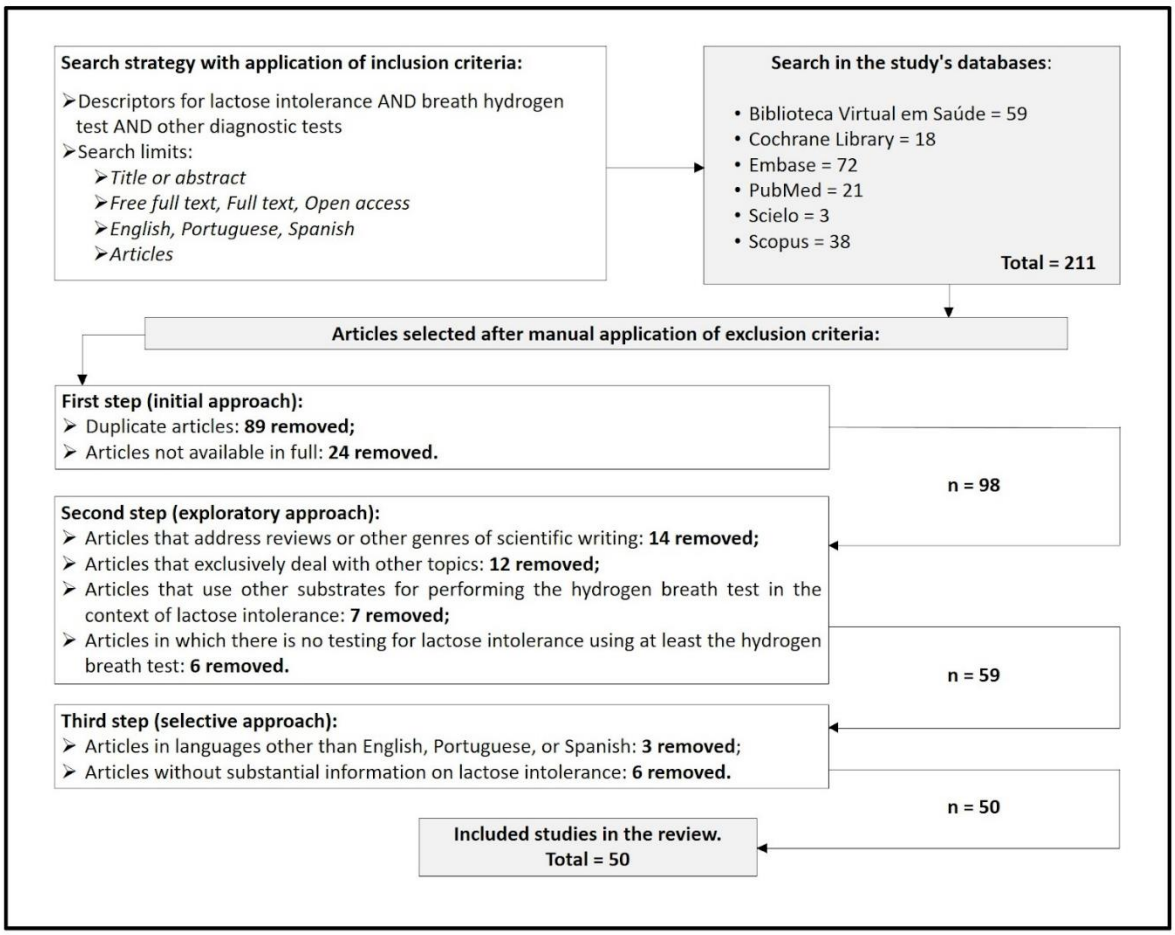

Figure 3. Flowchart of article selection (Source: the authors).

Table 1: Protocols for the use of hydrogen breath test for the diagnosis of lactose intolerance

Number of samples	Time between samples (gap)	Length of protocol	Dose of lactose	n	%	Article*
4 (1+3)	60 min.	180 min.	25.00 g	1	2,00%	(32)
4 (1+3)	60 min.	180 min.	50.00 g	1	2,00%	(63)
5 (1+4)	15 min. (0'-30'), 30 min. (30'-90')	90 min.	1.75 – 2.00 g/kg, up to 50.00 g	1	2,00%	(22)
5 (1+4)	30 min.	120 min.	25.00 g	2	4,00%	(26,45)
5 (1+4)	30 min.	120 min.	40.00 g	1	2,00%	(28)
5 (1+4)	60 min.	240 min.	2.00 g/kg	1	2,00%	(25)
6 (1+5)	30 min.	180 min.	2.00 g/kg, up to 50.00 g	1	2,00%	(57)
6 (1+5)	60 min. (0'-60'), 30 min. (60'-180')	180 min.	25.00 g	1	2,00%	(55)
7 (1+6)	15 min. (0'-90'), 30 min. (90'-120')	120 min.	18.00 g	1	2,00%	(56)
7 (1+6)	15 min. (0'-90'), 30 min. (90'-120')	180 min.	25.00 g	1	2,00%	(36)
7 (1+6)	30 min.	180 min.	1.00 g/kg, up to 25.00 g	2	4,00%	(23,50)
8 (1+6)	30 min.	180 min.	2.00 g/kg, up to 50.00 g	2	4,00%	(27,42)
7 (1+6)	30 min.	180 min.	25.00 g	3	6,00%	(49,58,62)
7 (1+6)	30 min.	180 min.	5-16 years: 1.00 g/kg, up to 50.00 g > 16 years: 25.00 g	1	2,00%	(38)
7 (1+6)	30 min.	180 min.	2.00 g/kg, up to 25.00 g	1	2,00%	(54)
7 (1+6)	30 min.	180 min.	50.00 g	2	4,00%	(43,51)
7 (1+6)	30 min.	180 min.	51.00 g	1	2,00%	(44)
8 (1+7)	15 min. (0'-90'), 30 min. (90'-120')	120 min.	50.00 g	1	2,00%	(60)
8 (1+7)	15 min. (0'-60'), 30 min. (60'-180')	180 min.	50.00 g	1	2,00%	(52)
9 (1+8)	30 min.	240 min.	0.50 g/kg, up to 25.00 g	2	4,00%	(33,41)
9 (1+8)	30 min.	240 min.	2.00 g/kg, up to 50.00 g	2	4,00%	(20,31)
9 (1+8)	30 min.	240 min.	20.00 g	1	2,00%	(47)
9 (1+8)	30 min.	240 min.	25.00 g	4	8,00%	(15,17,21,46)
9 (1+8)	30 min.	240 min.	50.00 g	2	4,00%	(18,53)
9 (1+8)	15-30 min.	240 min.	50.00 g	1	2,00%	(39)
10 (1+9)	15 min. (0'-30'), 30 min. (30'-180')	180 min.	1.00 g/kg, up to 25.00 g	1	2,00%	(29)
11 (1+10)	15 min. (0'-60'), 30 min. (60'-240')	240 min.	50.00 g	1	2,00%	(34)
11 (1+10)	15 min. (0'-120'), 30 min. (120'-180')	180 min.	50.00 g	1	2,00%	(61)
13 (1+12)	20 min.	240 min.	25.00 g	1	2,00%	(59)
14 (1+13)	10 min. (0'-60'), 20 min. (60'-180')	180 min.	50.00 g	1	2,00%	(30)
15 (1+14)	30 min.	420 min.	20.00 g	1	2,00%	(37)
17 (1+16)	15 min.	240 min.	50.00 g	1	2,00%	(48)
33 (1+32)	15 min.	240 min.	50.00 g	1	2,00%	(35)
Not specified	--	--	--	5	10,00%	(14,16,19,24,40)

*Numbers in this column correspond to the references of this review, cited in Vancouver format.

2 CONCLUSÃO

Este artigo objetivou analisar o emprego do teste de hidrogênio expirado para detecção de intolerância à lactose. Assim, em consonância com o fato desta tecnologia ter ganhado espaço, percebeu-se expansão de pesquisas na área ao longo do tempo.

Ademais, foi possível concluir que a literatura disponível de fato põe o teste de hidrogênio expirado como superior às demais abordagens diagnósticas muito em virtude de suas vantagens. Assim, embora apresente algumas limitações, estas apresentam pouca relevância para a prática clínica, de modo que é recorrente a consideração deste teste como novo padrão-ouro.

Contudo, cabe destacar certa fragilidade da literatura no que diz respeito à padronização da realização do HBT, havendo certa dissonância entre as pesquisas no que diz respeito às doses utilizadas e aos critérios diagnósticos relevantes para caracterização da intolerância à lactose.

Logo, embora tenha sido possível estabelecer uma base sobre o assunto, mais estudos na área precisam ser feitos de modo a consolidar o conhecimento acerca dos melhores protocolos de execução do HBT, seus valores diagnósticos, impactos adversos de dosagens adotadas e disponibilidades de aplicação clínica. Tais estudos serão importantes para validar uma abordagem diagnóstica concisa que leve a tratamentos adequados e eficientes.

ANEXO A – Normas do periódico Arquivos de Gastroenterologia

Regras gerais para publicação no periódico *Arquivos de Gastroenterologia* (ISSN 1678-4219 na versão digital), foram obtidas do site https://www.scielo.br/journal/ag/about/#instructions.

1. O texto deve estar no idioma inglês;
2. O número de autores é limitado a seis para os Artigos Originais;
3. Nenhuma taxa é exigida aos autores para submissão, avaliação e publicação de artigos;
4. O manuscrito submetido deve ser enviado em formato Microsoft Word e organizado da seguinte forma:
    a. Título em inglês e português;
    b. Nomes dos autores e suas afiliações. Não insira cargos, funções ou adjetivos;
    c. Para cada autor deve ser descrita em inglês a sua participação no estudo. As contribuições são, por exemplo: coleta de dados, execução de pesquisa, redação de texto, análise estatística etc.;
    d. Departamento e Instituição onde o trabalho foi realizado;
    e. ORCID de todos os autores;
    f. Declarar se há ou não conflito de interesse, subsídio ou outro apoio financeiro; os patrocinadores devem ser declarados;
    g. Resumo estruturado (Contexto, Objetivo, Métodos, Resultados, Conclusão). O Resumo deve ser enviado tanto em inglês como em português (de 200 a 600 palavras). Abreviações, notas e referências devem ser evitados;
    h. Palavras-chave (de 3 a 10). Utilize sempre que possível termos da lista Medical Subject Headings (MeSH) do MEDLINE. Informação disponível em: http://www.nlm.nih.gov/mesh/meshhome.html. A pesquisa também pode ser feita no portal Descritores em Ciências da Saúde, disponível em: https://decs.bvsalud.org/;
    i. Recomendamos a seguinte divisão dentro do artigo: Introdução; Métodos; Resultados; Discussão; Conclusão; Agradecimentos;

j. Todos os colaboradores que não sejam autores podem ser mencionados na seção de Agradecimentos;
k. Referências - A Arquivos de Gastroenterologia adota as normas Vancouver. Texto completo em: https://www.nlm.nih.gov/bsd/uniform_requirements.html;
l. Cite as referências no texto usando algarismos arábicos na ordem de citação, entre parênteses. Para até seis autores, todos devem ser citados. Para mais de seis autores, inclua "et al.";
m. Tabelas e Figuras devem ser citadas no texto em algarismos arábicos. De preferência, anexadas ao artigo em JPG ou PNG. Se estiverem dentro do artigo, devem vir ao fim, após as referências. Nunca devem ser colocadas no meio do texto.
n. Tabelas (em formato Microsoft Word ou Excel) - Intitula-se "Tabela" apenas quando há resultados numéricos. Explicações e abreviaturas devem ser colocadas em notas de rodapé da tabela;
o. Figuras – Nomeie como “Figura” sempre que for: questionário escrito, fotografias, gráficos e desenhos. Eles devem ser enviados em formato digital de alta resolução (2 mb). As figuras devem conter um pequeno texto sobre o assunto;

5. Recomenda-se uma carta de apresentação destacando a intenção de publicar no periódico Arquivos de Gastroenterologia e a importância desta pesquisa e publicação. Esta carta deve ser escrita no campo "Author’s Cover Letter" no cadastro on-line.