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Original article

Intestinal permeability in leukemic patients prior to chemotherapy

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ABSTRACT

Objective: The objective of this study was to evaluate the intestinal barrier function in leukemia patients before the start of the chemotherapy with an intestinal permeability test using lactulose and mannitol as markers.
Methods: The study enrolled 20 patients diagnosed with leukemia (acute and chronic). Ten healthy volunteers were also submitted to the test as a control group.
Results: The median lactulose/mannitol ratio was 0.019 for the Leukemia Patient Group, whereas in healthy controls the median was 0.009 (p-value = 0.244). The median lactulose/mannitol ratio in acute leukemia patients was 0.034 giving a p-value of 0.069 when compared to healthy controls. This same comparison was made between acute myeloid leukemia patients and healthy controls with a p-value of 0.149. There was no significant difference in the intestinal permeability between acute and chronic leukemia patients (p-value = 0.098).
Conclusion: The intestinal barrier function measured using the intestinal permeability test was similar in leukemic patients overall and healthy controls, but a tendency toward a different pattern was found in the intestinal barrier function of acute leukemia patients.

Introduction

Leukemias are diseases characterized by neoplastic proliferation that affect the bone marrow and inhibit hematopoiesis, causing abnormalities in peripheral blood and sometimes infiltrating non-hematopoietic tissues.[1] The gastrointestinal tract may be affected, either by leukemic infiltration or by therapy-associated complications.[2] Leukemia cell infiltration may occur in any segment of the gastrointestinal tract and may cause stomatitis, gingivitis or gum hypertrophy, oropharyngeal dysphagia and the formation of masses in the esophagus, stomach, small intestines and colon which, in turn, are associated to obstruction, hemorrhage,

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intussusception or enterocolitis. In the small gut, leukemia infiltration may reduce the integrity of the mucosal barrier, allowing antigen permeation and reduction of absorption area.[3] The involvement of this organ is most often seen in acute myeloid leukemia (AML).[2,4]

These changes in the intestinal barrier can be studied through intestinal permeability; the most commonly used markers are sugars such as lactulose and mannitol, since they do not require the use of radioactive techniques.[5–7]

The intestinal permeability test ($T_{L/M}$) is a useful method to evaluate the integrity of the intestinal mucosa in any condition which would cause the loosening of tight junctions, including those affecting the small gut, such as in Crohn's and celiac diseases and diseases which are associated to a secondary infiltration of this organ such as leukemia.[8] By using this method, it is also possible to study mucositis secondary to the use of chemotherapeutic agents.[9] Changes in intestinal permeability, detected before chemotherapy in leukemia patients and their eventual clinical consequences, such as a greater antigen permeation, may contribute to elucidate pathophysiological mechanisms involved in the context of the disease and its therapy, and possibly, come up with more specific solutions to problems related to these changes, such as infection and graft versus host disease (in the case of marrow transplantation).

Objective

The aim of the study was to evaluate the intestinal barrier function in leukemic patients prior to chemotherapy, by testing intestinal permeability by the determination of urinary lactulose and mannitol concentrations by high performance liquid chromatography (HPLC).

Methods

Patients

Between April 2010 and September 2011, this study enrolled 20 patients aged 18 years or above, of both genders, with initial diagnoses of AML, chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) admitted in the Hematology Outpatient Clinic and Emergency Department of the Hospital das Clínicas da Universidade Federal de Minas Gerais, before undergoing chemotherapy induction. The use of the $T_{L/M}$ did not result in any change in medical management. Ten over 18-year-old healthy volunteers, of both genders, also underwent the $T_{L/M}$.

Patients diagnosed with bowel disease, cirrhosis, congestive heart failure, nephrotic syndrome, thyroid diseases or diabetes mellitus, diseases that could interfere with absorption or flow of water and solutes and/or gastrointestinal motility were excluded from the research as were patients who drank alcoholic beverages within three days and took non-steroidal anti-inflammatory drugs (NSAIDs) within seven days prior to urine collection.

Methods

The study was approved by the Research Ethics Committee of the Universidade Federal de Minas Gerais (ETIC 0079.0.203.000-11). All participants signed an informed consent term before the study was initiated.

Diagnosis of leukemia was confirmed by myelogram, bone marrow biopsy and cytogenetic or genetic studies when necessary.

In order to perform the $T_{L/M}$, patients fasted for eight hours. Subsequently, they were instructed to eliminate any residual urine and a 120 mL iso-osmolar solution containing 6.25 g of lactulose (95%) (Sigma–Aldrich, Missouri, USA) and 3.0 g of mannitol (PA) (Sigma–Aldrich, Missouri, USA) diluted in water was given. Fasting was maintained for the following two hours. All urine volume was collected during a period of five hours. Subsequently, the urine was homogenized and the total volume was recorded. Aliquots of 50 mL were stored in labeled in sealed flasks after adding 10 mg of thimerosal (Synth, Diadema, Brazil) to inhibit bacterial growth. Samples were filtered using a millipore filter (0.22 μm) (Millipore, Billerica, USA), and the ion-exchange resin and the material were stored in properly labeled cryotubes at −20 °C.

The mannitol and lactulose concentrations were measured in the urine using HPLC equipment (Schimadzu®, Japan) comprising an injection pump, an autoinjector, a controller with software that allows readings to be interpreted at a workstation, and a refractive index gauge. Fifty microliters of urine were introduced after thawing using the autoinjector. To achieve better separation from other substances in the urine, lactulose and mannitol were read using two different columns utilizing two distinct mobile phases. A Phenomenex H^+ column (Phenomenex, USA) with a mobile phase of pure milli-Q sonicated water at a flow of 0.6 mL/min was used to separate the mannitol and a Supelcogel NH_2 column (Sigma Aldrich, Bellefonte, USA) with a mobile phase of a solution of acetonitrile and milli-Q sonicated water (ratio of 75/25) with a flow of 1.0 mL/min was employed to separate the lactulose. A Supelcogel H^+ precolumn (Sigma Aldrich, Bellefonte, USA) was the same for both readings. Different amplitudes of the waves generated by the solution containing lactulose and mannitol were captured at the workstation, generating graphs in the form of curves, which were then recorded. Analyses were carried out at room temperature.

To test reproducibility and to standardize measurements, solutions of lactulose were prepared at known concentrations of 0.1 g/L, 0.2 g/L, 0.4 g/L and 0.8 g/L, as were solutions of mannitol at concentrations of 0.625 g/L, 1.25 g/L and 2.5 g/L and a simple linear regression was performed in order to obtain a straight line equation for both.

By correcting for the urine volume, the amount excreted was obtained for lactulose and mannitol, which was then divided by the amount ingested to calculate an excreted percentage of each sugar. The percentage of lactulose was divided by the percentage of mannitol in order to obtain the lactulose/mannitol excretion ratio ($T_{L/M}$).

Clinical and laboratory variables were recorded. Statistical analyses were performed using the Statistics Program for Social Sciences (SPSS version 18.0). Student's *t* test was employed to compare independent sample means and when