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Role of glycated hemoglobin in the screening and diagnosis of posttransplantation diabetes mellitus after renal transplantation: A diagnostic accuracy study

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ABSTRACT

Background: The role of glycated hemoglobin (A1C) in the screening and diagnosis of posttransplantation diabetes mellitus (PTDM) is still not entirely understood. We evaluated the use of A1C test in renal transplant recipients at four months after transplantation.
Methods: A total of 122 out of 274 patients without previous diabetes that underwent kidney transplantation were enrolled. ROC curve was used to analyze the performance of A1C to diagnose PTDM considering OGTT as the reference standard.
Results: OGTT identified 32 (26.2%) patients with PTDM, whereas A1C ≥ 6.5% (48 mmol/mol) identified only 16 patients. A1C showed moderate accuracy to detect PTDM in the ROC curve [AUC 0.832 (95% CI 0.740–0.924, $p < 0.001$)]. A1C of 5.8% (40 mmol/mol) was the equilibrium point (sensitivity 75% and specificity 72.2%) and A1C ≥ 6.2% (44 mmol/mol) showed high specificity of 93.3%.
Conclusions: A1C ≥ 6.5% (48 mmol/mol) is not enough to be used alone in the diagnosis of PTDM. The combined use of A1C cut-off points of ≤5.8% (40 mmol/mol) and ≥6.2% (44 mmol/mol) would reduce the number of OGTT by 85%. The use of an algorithm with A1C test in combination with FPG and/or 2h-PG proved to be the most efficient strategy to diagnose or rule out PTDM.

1. Introduction

Posttransplantation diabetes mellitus (PTDM) has been recognized as an ominous complication of renal transplantation; its incidence during the first year after transplant ranges between 10% and 30% [1]. Immunosuppressive therapy, usually including steroids and a calcineurin inhibitor, is a major risk factor for the development of abnormalities in glucose metabolism after transplantation. In susceptible individuals, their use is associated with the development of insulin resistance and/or the decrease of insulin secretion [2]. An adequate screening of these metabolic changes is important in order to avoid poor outcomes related to hyperglycemia [3].

In 2003, the International Consensus Guidelines on New Onset Diabetes After Transplantation established a standard definition for diagnosing PTDM [4]. According to these guidelines, the diagnosis of PTDM should follow the same criteria recommended by the American Diabetes Association (ADA) and by the World Health Organization (WHO) for the diagnosis of diabetes mellitus (DM) in the general population. At the time of publication, diagnostic criteria were based on glucose based-tests: random blood glucose (RBG) and/or fasting plasma glucose (FPG) and/or 2-h plasma glucose after 75-g glucose (2h-PG) by an oral glucose tolerance test (OGTT) [5]. In 2010, ADA guidelines also included glycated hemoglobin (A1C) as diagnostic criterion, considering A1C ≥ 6.5% (48 mmol/mol) as the cut-off for the diagnosis of DM [6,7].

Although A1C test is useful to detect DM in the general population, there are still controversies about the applicability of its results within the initial months post-transplantation. In this period the need for dialysis treatment, anemia and the use of recombinant erythropoietin are all factors that may affect A1C results and lead to misinterpretations [8,9].

Abbreviations: ADA, American Diabetes Association; PTDM, posttransplantation diabetes mellitus; STARD, Standards for Reporting of Diagnostic Accuracy; 2h-PG, 2-hour plasma glucose after 75-g glucose.

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Recently, a consensus meeting on PTDM discussed diagnostic challenges surrounding the development of PTDM and the importance of the use of different tests to improve screening strategies [10]. According to these meeting recommendations, A1C between 5.7% and 6.4% (39–46 mmol/mol) may be indicative of metabolic changes and require further investigation with a glucose based-test. Furthermore, A1C ≥ 6.5% (48 mmol/mol) is highly specific for the diagnosis of PTDM, as well as for DM in the general population, and is unlikely to be a false positive [10]. Previous studies that investigated A1C levels as a tool for diagnosing PTDM led to controversial results [11–15]. Discrepant sensitivities were observed at A1C cut-off point of 6.5% (48 mmol/mol), and there was no agreement on which cut-offs are ideal either to indicate the need for an OGTT or to confirm the diagnosis of PTDM.

So far, few papers have discussed the associations between PTDM and microvascular and macrovascular complications [16,17]. FPG levels have been shown to be lower in renal transplant patients than in subjects from the general population with similar OGTT results (impaired glucose tolerance and overt diabetes) [18]. The consensus meeting on PTDM suggests a reduction in FPG threshold for the PTDM screening. It also suggests the use of ADA cut-off (100 mg/dl) over the WHO cut-off (110 mg/dl) to identify patients with increased risk of diabetes [10]. For A1C such a recommendation cannot yet be made, and a previous paper [13] advocating an A1C cut-off point of ≥6.5% (48 mmol/mol) is seemingly in disagreement with another recent publication [15] advocating a lower cut-off. In order to help clarify these issues, we evaluated the use of A1C test to diagnose PTDM and assessed the overall accuracy of the A1C test in renal transplant recipients at four months after transplantation considering FPG ≥ 126 mg/dl and/or 2h-PG ≥ 200 mg/dl during an OGTT as the reference diagnostic test.

2. Materials and methods

2.1. Study design

The study followed the Standards for Reporting of Diagnostic Accuracy (STARD) Initiative [19]. A cross-sectional study design was used to evaluate the diagnostic accuracy and effectiveness of the A1C test in diagnosing PTDM at four months post-transplantation. The research design considered the PTDM diagnosis as defined by New Onset Diabetes After Transplantation Consensus Guidelines: FPG ≥ 126 mg/dl and/or 2h-PG ≥ 200 mg/dl [4].

2.2. Study population

The study included adult patients without DM that underwent kidney transplantation at Hospital de Clínicas de Porto Alegre (HCPA), a tertiary university teaching hospital in southern Brazil, between March 2012 and April 2014. All patients were invited to participate and to undergo an OGTT according to WHO recommendations [20]. Exclusion criteria were: presence of anemia (hemoglobin < 11 g/dl for men and <10 g/dl for women) [21], estimated glomerular filtration rate (eGFR) of <15 ml/min [22], presence of variant hemoglobins in the A1C chromatogram [23], use of recombinant erythropoietin [24], dialysis treatment or blood transfusion within the previous three months [25,26], and having PTDM diagnosed before the evaluation period. Patients with DM prior to the transplant were also excluded based on medical history and on FPG levels as established by ADA criteria [27].

Once participants were selected, the following data were recorded: age, race, gender, blood pressure, waist circumference, donor type, immunosuppressive medication, acute rejection episodes, family history of DM and immunosuppressive drug blood levels. Body weight and height were measured and BMI (kg/m^2) was calculated for all patients.

All participants signed an informed consent form prior to their inclusion in the study, and answered a standardized questionnaire for demographic information. The research protocol was approved by the Research Ethics Committee of HCPA; followed the principles of the Declaration of Helsinki.

2.3. Immunosuppressive therapy

To reduce factors affecting A1C results in the early post-transplant period (i.e. anemia, blood transfusion, hemodialysis) and to include patients with steady doses of immunosuppressive drugs, we arbitrarily included patients at the beginning of the fourth post-transplant month. Maintenance immunosuppression at this time point comprises, for all patients, low dose prednisone (5 mg), a calcineurin inhibitor (tacrolimus or cyclosporine), and mycophenolate mofetil/sodium. Cyclosporine was given mainly if tacrolimus was withdrawn due to the occurrence of significant side effects. All patients received a 500 mg dose of intravenous methylprednisolone at surgery. At post-operative day one, they were started with oral prednisone 20 mg/day, which was tapered by 5 mg/day, per month, until they reached a final dose of 5 mg/day at the beginning of the fourth month post-transplantation. Target tacrolimus trough levels were 10–12 ng/ml for the first two months post-transplantation and between 8 and 10 ng/ml for the third and fourth months post-transplantation. Target cyclosporine trough levels were 200–300 ng/ml for the first two months and between 200–250 ng/ml for the third and fourth months post-transplantation.

2.4. Laboratory analysis

All laboratory measurements were carried out at HCPA Clinical Pathology Department. After an overnight fast, a standard OGTT was performed and blood samples were drawn to determine levels of A1C, cell blood counts, creatinine, and glucose. Plasma glucose was determined by enzymatic method (Advia® 1800, Siemens Healthcare), cell blood counts were estimated by flow cytometry (Advia 2120i, Siemens), and creatinine was measured by Jaffe reaction (Advia® 1800, Siemens). The eGFR was estimated by the CKD-EPI equation as validated for renal transplant recipients at our institution [28]. A1C was measured by the HPLC method (Bio-Rad Variant™ II Turbo analyzer), as standardized by the National Glycohemoglobin Standardization Program (NGSP; http://www.ngsp.org/certified.asp) and aligned with the International Federation of Clinical Chemistry (IFCC) [29]. The Clinical Pathology Department is an A1C External Quality Assurance Program participant with excellent performance.

2.5. Statistical analysis

Data were expressed as mean ± standard deviation (SD), median (interquartile range) or frequencies (%). When appropriated, Student's *t*-test, Mann–Whitney U test and chi-squared test were used to analyze differences between groups with and without PTDM. The ROC curve was used to analyze the performance of A1C test to diagnose PTDM considering FPG and/or 2h-PG after an OGTT as reference diagnostic criteria. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (LR) for different A1C cut-off points were calculated. A LR greater than 1 for a positive test was associated with the presence of the disease (LR+) and a LR less than 1 for a negative test was associated with the absence of the disease (LR−) [30]. For clinical practice applicability, we also applied the Bayes theorem to estimate the post-test probability of PTDM by using the Fagan nomogram [31], considering the pre-test probability of 20%, estimated from the literature [32,33]. A $p < 5\%$ was considered significant. The minimum sample size needed for this study, to obtain an area under the curve (AUC) of at least 0.80, with 90% of power and 5% of significance, were 17 patients with PTDM and 17 without PTDM (R-project/pROC).