Diabetes Mellitus Classification

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Abstract

The right classification for diabetes mellitus (DM) allows a more adequate treatment and comprises four categories: type 1 DM, type 2 DM, other types, and gestational diabetes. In some cases, there might be a superposition of situations, especially with regard to the DM that initiates in the young adult or is initially presented with diabetic ketoacidosis intermediately to type 1 and 2 DM. Thus, additions to the classic classification system have been proposed as assessing the presence of autoimmunity (antibody) and b cell function (C-peptide) to precisely define the subtypes. The aim of this literature review was to analyze these diagnostic indexes' performance in the DM classification and to describe subtypes with details. The antibodies against pancreas confirm autoimmunity, and the antibody against insulin is more accurate before 5 years old, while the *anti-glutamic acid* decarboxylase is more accurate after 20 years old, a test which remains positive for a longer period. The measurement of C-peptide evaluates the pancreatic insulin reserve, and the most largely used methods of stimulation are the measurement after meals or after *intravenous glucagon. C-peptide values* < 1.5 ng/ml define a patient with absent pancreatic function and, above this value, patients with preserved function. When the presence of antibodies (A+) directed to the pancreas is combined to its insulin secretion capability (β+), it is possible to subdivide DM's classification in type 1A (A+β-) and 1B (A+β-); and type 2A (A+β+) and 2B (A-β+), which allows a more precise classification and treatment besides opening horizons for the understanding of DM pathogenesis.

Diabetes mellitus (DM) is a common disease which is considered epidemic by World Health Organization (WHO). Estimative for world prevalence is around 4.0%[1,2] and, in Brazil, around 7.6%, as shown in the last evaluation[3]. Its incidence in adults and adolescents have been alarmingly rising in developed countries with estimatives for an increase of 60% in the adult population with more than 30 years old in 2025[1], as the higher prevalence would be present in 45 to 64 years-old adults[1,2].

Key words

Diabetes mellitus/classification; glutamic acid; carboxy-lyases; C-peptide.

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Manuscript received in October, 13 2008; revised manuscript received in February, 16 2009; accepted in May, 6 2009.

The right classification for DM type allows a precocious adequate treatment, with higher rate of success concerning the obtainment of a safe glycemic control, which reduces microvascular complications in patients with type 1 or type 2 DM[4,5]. Although randomized clinical trials have not shown reduction in cardiovascular outcomes during intensified glycemic control studies, the long term follow-up of patients have shown a decrease in the risk of macrovascular disease[6]. Currently, the American Diabetes Association (ADA) and the American Heart Association (AHA) recommend that the target range for HbA1c be in general < 7% (ADA: B level and AHA: A level). Less rigorous target ranges have been suggested for individuals with history of severe hypoglycemia, limited expectation of life, advance-staged micro or macrovascular disease and important comorbidities (ADA: C level and AHA: C level).

New DM classification was redefined in an ADA's publication in 1997[7] and of WHO in 2006[8]. Updated national and international directions recommend DM's classification in four categories: type 1 DM (DM1), type 2 (DM2), other types and gestational diabetes[9] (Chart 1).

DM1 is responsible for approximately 5 to 10% of all DM cases, and is subdivided in type 1A, type 1B and Latent Autoimmune Diabetes of the Adult (LADA). In general, DM1 is disclosed before 30 years old, but may attain individuals of all age groups. There is a destruction of pancreatic β-cells, and its treatment requires insulin for hindering diabetic ketoacidosis[3]. In DM 1A, β-cells destruction is of autoimmune cause (90% of cases) and in DM 1B there are no known causes (idiopathic). LADA is a type of DM1 in which autoimmune β-cells destruction also happens, but it is much slower and comprises older individuals (older than 30 years old). its phenotype is peculiar, for patients are not obese, had DM diagnosed in age compatible with type 2 DM, are initially controlled by oral agents, but they present signs of progressive β-cell's function loss[9] and eventually need insulin after at least six months post-DM diagnosis[9]. DM 1B was initially described in Africans and Asians[7]. However, this type have been assessed and described with more details in other populations, which brought out a new nomenclature: ketosis-prone DM[10], a type of DM that would be intermediate to type 1 and 2. These cases would differ from LADA for being initially manifested as ketoacidosis, while proper LADA requires insulin six months after diagnosis. An additional observation describes the presence of antibodies in children and adolescents that are not dependent on insulin initially, but present DM2 profile. In these cases, as following LADA terminology for adults, the right classification would be "LADY" (Latent Autoimmune Diabetes in Youth), but a

Chart 1 - Etiologic classification of diabetes mellitus

I. Type 1 diabetes
A. Immunologically mediated
B. Idiopathic
II. Type 2 diabetes
III. Other specific types
Genetic disorder of β-cell function (MODY, mitochondrial DNA)
Genetic disorders in insulin action (lipoatrophic diabetes)
Exocrine pancreas diseases (pancreatitis, hemochromatosis)
Endocrinopathies (acromegaly, Cushing's syndrom)
Drug-induced (*glucocorticoids*, tiazidics)
Infections (cytomegalovirus, congenital rubeola)
Uncommon immunological forms (insulin receptor antibodies)
Other genetic syndrome (Down, Turner, Prader-Willi syndrom)
IV. Gestational diabetes

Source: adapted from American Diabetes Association[9].

longer period of follow-up is still necessary for defining such cases' development, besides the fact that this nomenclature is not official[11].

DM2 is responsible for more than 90% of DM cases, has no autoimmune component, and is unleashed generally after 30 years old in individuals with positive family history. The initial treatment usually involves diet and oral hypoglycemic agents with no need for insulin. When necessary, insulin can be added to treatment, but this usually happens, at least, five years after the diagnosis configuring a initial state of non insulin requiring diabetes, the opposite of type 1 DM[6,7].

In the "other DM type" category, the Maturity Onset Diabetes of the Young (MODY) is highlighted, a subtype that attains individuals younger than 25 years old, non-obese, and which is characterized by a deficiency in insulin secretion but does not provoke dependence on it. It also presents dominant autossomic inheritance, thus involving many generation of one sole family[7,12]. There are six subtypes of MODY, which are qualified according to the identified genetic mutation: MODY 1 (mutation in the *hepatic transcription factors gene, HNF-4α*); MODY 2 (mutation in the glycokinasis gene); MODY 3 (mutation in the HNF-1α gene); MODY 4 (mutation in the insulin promoter factor, also known as IPF); MODY 5 (HNF-1β) and MODY 6 (mutation in Neuro-D1 gene). The more common types are MODY 2 and 3, and the others are very rare[12].

Unlikely MODY, that typically represents a pattern of monogenic DM, DM2 brings a polygenic inheritance that has not been completely defined yet. DM2's pathogenesis is complex and comprises the interaction between genetics and environment factors, especially obesity due to sedentary life and to excessive food intake. With regard to DM2's genetic aspects, some genes, such as calpain-10, PPARγ2 and Kir6.2, have been confirmed, but it is necessary to go forward in the elucidation of these and other genes' roles[13]. Although DM1 participate in the autoimmune 1A subtype, it is a heterogeneous disease and many patterns of monogenic inheritance (associated with autoimmune polyendocrinopathy) have already been defined. Nonetheless, the majority of the genes that promote genetic susceptibility in type 1 are still to be identified.

Despite the fact that this classification defines this and other categories through peculiar characteristics, there might be a superposition of clinical situations, mainly concerning DM that develops in the young adult. Sometimes it is difficult to distinguish a subtype from another based solely on clinical data, especially DM1 MODY and DM2 of precocious initiation, which is becoming more and more frequent due to an increase in obesity and metabolic syndrome prevalence among occidental societies. Besides, some types of DM identified in the last decades, which are intermediate to type 1 and 2, do not comply with ADA and WHO's classifications, for there are particularities in their natural history, as well as the necessity of insulin treatment. The aforementioned DM propense to ketosis is an example[11].

For that reason, complementary schemes for classification have been proposed with the involvement of autoimmunity (antibodies) and of β-cells function indexes (C-peptide) capable of not only defining more specific pathogenesis and nomenclature, but also suggesting more adequate methods of treatment. The Aβ classification (A=antibodies and β=islet function) has been proposed with the purpose of complementing the traditional ADA classification for the types of diabetes which have propensity to ketosis, but without being contradictory to it. The presence of antibodies is defined as "A+" while the preservation of pancreatic function is defined as "β+". ADA's classification or DM1 cases is maintained in two subgroups (type 1A and 1B, with and without antibodies, respectively, and both without insulin production), and this subdivision is applied to DM2, which implies having insulin reserve with or without antibodies.

This proposal is based on the recognition of DM heterogeneous syndrome, which have been identified and followed in cohorts. Through the comparison between other classifications, it has shown to be more accurate and predictive of important outcomes, such as glycemic control and dependence on insulin, as well as a good indicator of new causes for β-cell dysfunction, with molecular and biochemical profile already described[11].

The objective of this literature review is to assess the performance of the indexes that evaluate autoimmunity and insulin secretion with the purpose of putting DM into classification and describing in details the subtypes that were generated by such classification.

The role of pancreatic antibodies

Autoimmunity against pancreatic islets was described in 1965, but the presence of antibodies (AB) against islets (the islet-cell cytoplasm antibodies; ICA) was first demonstrated in 1974[14]. Following, the existence of many other antibodies was identified: islet cell surface antibody (ISCA); insulin auto-antibodies (IAA); and insulinoma like antigen-2 (IA-2). The different types of antibodies directed to the pancreas as well as their role in the diagnosis of patients with DM1 will

be herein described (Chart 2)[16].

The presence of antibodies indicates a DM of autoimmune etiology and, thus, type 1 classic, which is called type 1A. Many methods were created, in international collaborations, to measure the diverse antibodies and to standardize it[17,18]. As there is no international standard for the islet-cell antibody and also a lack of studies with consistent and reproducible results, its use is limited to research[14]. The islet-cell antibodies, the *anti-glutamic acid* decarboxylase and the insulinoma like antigen-2 were subject for trials based on recombinant proteins, which may be marked with radioactive iodine, allowing the development of reproducible and precise trials that are already standardized according to the criteria recommended by WHO[17]. The sensibility and specificity for the diagnosis of DM1 are presented in Chart 2.

The islet-cell antibodies, initially described in 1974[13], were the first ones to be used, but their laboratorial standardizing happened only in 1986[19]. Their reaction occurs against all components of pancreatic islet[14,15]. There are several other pancreatic islet antibodies and many are still to be recognized. However, the *anti-glutamic acid* decarboxylase, anti-insulin, anti-islet and AI-2 have more utility in the clinical practice[20].

The islet-cell antibodies are efficient markers of DM1. Their sensibility varies from 70 to 90%[16,17]. However, the capability of predicting risk for DM1 decreases significantly in individuals with symptoms manifestation in advanced ages, mainly after 20 years old[21,22]. These antibodies are present during the pre-diabetes phase and in the beginning of the disease establishment, but their titles drop rapidly right away[21,23]. Their presence is associated with a rapid loss of β-cell function[21] and with the prediction of insulin necessity in patients initially diagnosed with DM2[21,23,24].

Anti-insulin antibodies are present in approximately 50% of patients recently diagnosed with DM1, and they are reckoned by means of a radioimmune trial of simple liquid phase[25]. It shows 50 to 70% of sensibility in the DM1 diagnosis. In general, the younger is the patient in the beginning of the disease, the bigger is its positivity[26]. It consists of a efficient marker for pre-clinical disease in children especially those who are less than 5 years old, for it predicts DM risk in children better than in adults[22,27].

The *anti-glutamic acid* decarboxylase antibody was initially described as a protein of 64 kilodaltons[14,28]. There are two isoforms[29]: one denominated 65 (expressed in β-cells) and other denominated 67 (in the brain). The function of *glutamic acid* decarboxylase enzyme consists of producing the inhibitory neurotransmitter GABA and, in the pancreatic β-cell, *adenosine triphosphate (ATP)*[29]. Anti-*glutamic acid* decarboxylase antibody's sensibility for DM1 diagnosis is between 75 and 85%[30]. There is a great difference in its sensibility concerning genre[31] and age of DM diagnosis[30]. For females, its sensibility is around 80% and does not vary with age. For males, the sensibility of approximately 50 to 60% before 10 years old and between 75 and 90% after this age[30,31]. Specificity reaches 99% and the sensibility is considered as better than anti-islets and anti-insulin in adults[30,32]. The *anti-glutamic acid* decarboxylase is the most durable antibody, for it may still be positive after 15 of the establishment of DM[33]. In first-grade relatives, its positivity varies from 6 to 8%, similarly to the risk of DM1 development during life[30]. It is more positive in sons of fathers than in sons of mothers that are carriers of DM1[34]. In Diabetes Prevention Trial (DPT-1)[35], this antibody was the most sensitive marker for the detection of multiple antibodies. However, it is known that no antibody predicts solely and satisfactorily the risk of DM1 development, which is related to the number of antibodies present during the autoimmune evolution process[35-38]. The risk of DM development in first grade relatives carriers of DM1 was 39 to 68% after three and five years, respectively, when two antibodies were present. The presence of three antibodies shows a 100% risk within five years[36].

IA-2, also known as ICA-512, is a protein expressed in the neuroendocrine tissue and that is present in pancreatic α and β-cell. The most accurate measure trials are radioimmune trials[39]. Their sensibility for DM1 diagnosis varies from 60 to 70%. Positivity decreases according to the disease's duration, and is higher in patients diagnosed with DM before 10 years old.

In short, antibodies are autoimmunity markers and their presence means type 1 diabetes. In children with DM1 there is positivity for the presence of antibodies in 90% of the cases. Anti-insulin antibody is present in younger individuals, especially those who were diagnosed with DM before 5 years old, and it is considered the best marker of the disease in this age group.

The *anti-glutamic acid* decarboxylase shows a better performance in individuals that had manifestation of the disease after 20 years old. It is also the one that remains positive during longer periods (10 to 15 years of disease), and is often chosen for LADA diagnosis. Positivity of antibodies predicts the insulin necessity and their solicitation is indicated in cases of diagnostic doubt, that occur mainly when DM is established after 30 years old (Figure 1).

Chart 2 - Diagnostic performance of main antibodies against pancreas for DM 1 classification

Antibody	Sensitivity (%)	Especificity (%)
Anti-islet	70 to 90	96 to 99
Anti-insulin	50 to 70	99
Glutamic acid decarboxylase	70 to 90	99
Insulin-like antigen	55 to 75	

Source: adapted from Verge et al[31].

The role of C-peptide

C-peptide levels (CPEP) have been employed as β-cell function indexes, considered as an auxiliary exam in classification of DM type and for the initial choice of treatment[40]. Preservation of endogenous secretion is correlated to a precise glycemic control[41-45], reduction of DM chronicle complications and of hypoglycemic episodes[41,46]. CPEP presents as advantage the fact that, unlikely insulin, it is not degraded by the liver, but is exclusively eliminated by the kidney and has a 30-minute half-life[47]. Besides that, exogenous insulin interferes with endogenous insulin dosage, while CPEP

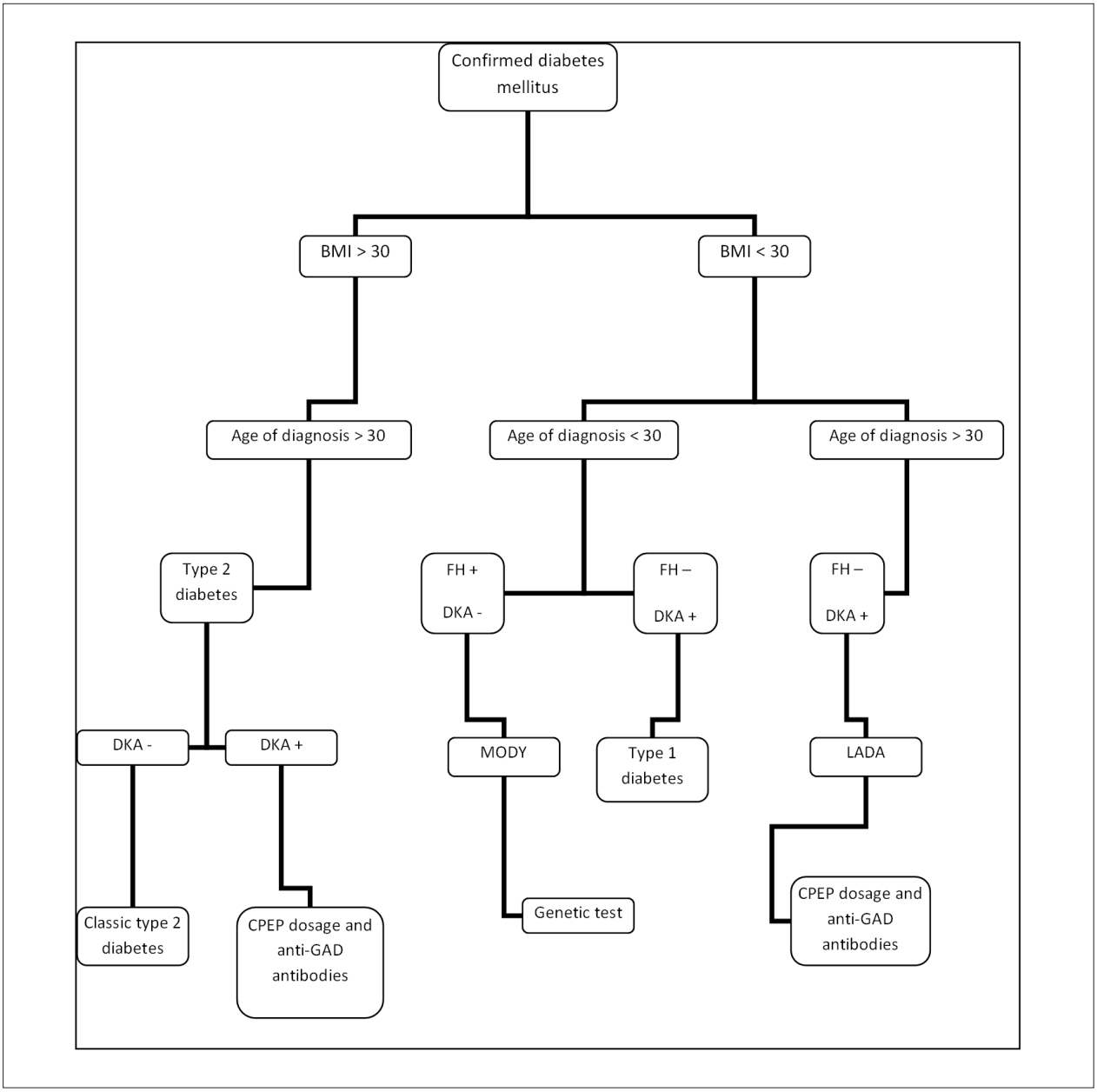

Figure 1 - Algorithm for diabetes mellitus classification. BMI - body mass index (kg/m²); FH - family history; DKA - diabetic ketoacidosis; LADA - Latent Autoimmune Diabetes of the Adult; MODY - Maturity-onset Diabetes of the Young.

does not suffer this intervention.

The two-chain insulin structure was first described in 1955[48] and, in 1967, the existence of a biosynthetic precursor was documented: the proinsulin[49]. The CPEP is a subproduct of proinsulin degradation and is co-secreted with the insulin by the pancreatic β-cell. Inside the pancreatic islet, proinsulin suffers cleavage, generating insulin and CPEP as final products, which are liberated in the portal circulation in equimole concentrations (Figure 2)[50].

CPEP consists of a small molecule that may suffer cleavage from *proteolytic enzymes* and, therefore, the plasma must be soon separated (within less than two hours), which should be carried out during the first freeze month. World strategies for the standardization of CPEP measurement are being currently considered[46,51].

One of the most important aspects, which have not been well established yet, concerns the glycemic homeostasis conditions under which CPEP must be measured[52]. Hyperglycemia may increase (by direct stimulation of glycemia) or decrease (by glucotoxicity) the β-cell response to the test, as well as hypoglycemia may inhibit the β-cell response[52,53]. For that reason, CPEP measure must be made in the absence of hyper or hypoglycemia, as the ideal glycemia would be between 70 and 200 mg/dl.

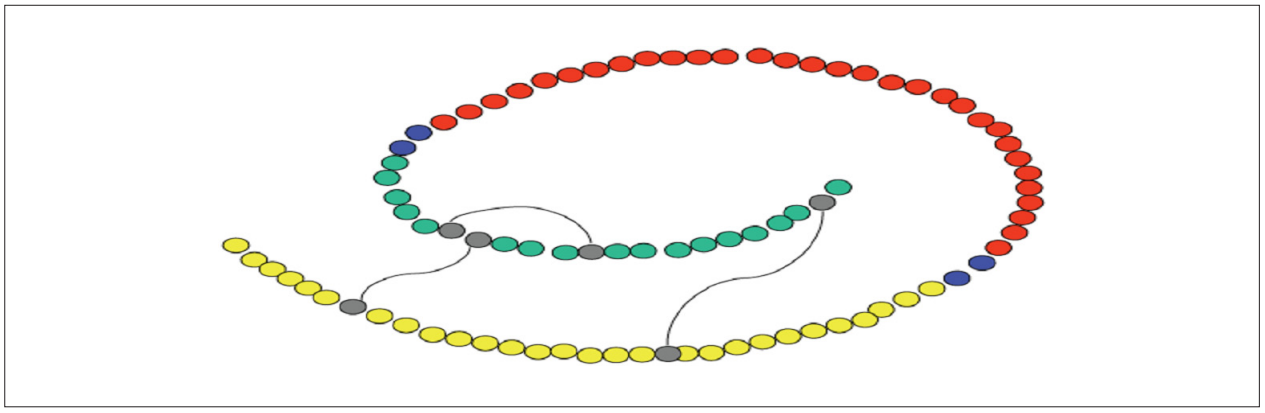

Figure 2 - Structure of human proinsulin. Insulin's A (green) and B (yellow) chains linked by C-peptide (red).

CPEP measure may be made in the basal or stimulated by endovenous, intramuscular or subcutaneous glucagon; oral or endovenous amino acids, oral or endovenous glucose; and by mix diet[54-57]. The two most used stimulations are endovenous glucagon and the mix diet test, as the latter is strongly recommended by ADA.

The patient is characterized with DM1 when presents CPEP values inferior to 1.8 ng/dl after 1 mg of endogenous glucagon[58], and inferior to 1.5 ng/dl after the mix diet test[45]. Some authors suggest that the basal value alone would be sufficient to characterize the patient, and a study have shown that the random CPEP value (1.5 ng/ml cut-point) measured at any time would be more sensitive[59]. A classical Denmarkian study indicated that a basal value inferior to 0.9 ng/ml could already be able to indicate insulin dependence[47]. Many authors do not take into account the duration time effect of DM in CPEP levels, once several other studies that followed diabetic patients have showed that the β-cell function decreases together with the duration of DM, and is faster in patients with a bad glycemic control[41,45,60,61]. As the decreasing rate varies, one single measure is enough for the diagnosis in new cases, but not to evaluate its decrease and, thus, repeated measurements are recommended[46,60,62].

In summary, the CPEP measure appears to be a good marker for the β-cell function and must be made with glycemia between 70 and 200 mg/dl. Stimulation with mix diet is recommended by ADA, but the test performed with endovenous glucagon 1 mg is simpler and equally effective. Stimulated values that are inferior to 1.5 ng/ml define the DM1 patients, while the superior values define the DM2 patients (Figure 1).

Critical analysis of classification schemes

DM is defined as a heterogeneous group of metabolic diseases, and its correct classification must be based on physiopathological principles and be helpful in the choice of the treatment[7,8]. However, current sudies[63,64] show that the available classification scheme does not truly reflect all the involved mechanisms. Some DM subtypes have been described in the last decades. In 2002, the name "Ketosis-prone diabetes" was created, which would correspond to a subtype that is intermediate to type 1 and 2[10]. Initially, the case was described in Africans and Afro-Americans, but in the past few years American, European, Japanese and other populations have presented this subtype[63-65].

It is known that DM2, in stress situations such as severe infections and cardiovascular diseases, may present with ketoacidosis. In contrast, the ketosis-prone diabetes manifests initially with ketoacidosis symptoms without apparent causes, as the name says. Finally, the non-ketosis-prone diabetes is the classic DM2, which does not cause dependence on insulin.

There are four different classifications for ketosis-prone diabetes. The ADA's scheme[7], other scheme modified from ADA's classification[65], the scheme based on body mass index (BMI)[66] and the one that assesses the presence of autoimmunity and the β-cell function, called Aβ[64].

According to ADA's classification, the presence of ketoacidosis characterizes the person as a DM1 patient, and it is considered subtype 1A if there are any positive immunologic markers against β-cell, and type 1B (idiopathic) if they are absent.

The modified ADA's classification[65] divides patients into three groups. Type 1 has positive immunologic markers against β-cells. Those that do not present antibodies are divided into two subgroups: dependent and independent on insulin, a classification based on the necessity of insulin at long term. In general, type 1A and insulin-dependent diabetes have clinical characteristics of DM1 with low β-cell function, while the insulin-dependent types have clinical characteristics of DM2 with preserved β-cell function[65].

The scheme based on BMI[66] characterize the patients as thin (BMI < 28 kg/m^2) or obese (BMI > 28 kg/m^2). The thin patients present DM1 with low β-cell function characteristics, and the obese patients present DM2 with preserved β-cell function characteristics.

The classification Aβ[64] is based on the presence of autoimmunity markers against pâncreas (A+ ou A-) and associated with the analysis of the β-cell function (β+ ou β-). There are four groups: type 1ª DM, with positive autoimmunity and absence of β-cell function (A+β-); type 1B DM, without autoimmunity and absence of β-cell function (A-β-). These first subtypes are comprised in ADA's classification. Additionally, two more subtypes are described as branches of DM2: DM2

with autoimmunity and preserved β-cell function (A+β+), and DM2B, without autoimmunity and maintained β-cell function (A-β+). The fluxogram indicates how to identify these subtypes (Figure 1).

A+β- and A-β- patients are immunologically and genetically different from one another, but they present DM1 clinical characteristics with low β-cell function. A-β+ and A+β+ patients are immunologically and genetically different, but with DM2 clinical characteristics and preserved β-cell function[64]. Therefore, there are important differences in phenotypes and in natural history of subtypes that remain unexplained in ADA's classification, in which both β- subtypes would be DM1 and both β+ subtypes would be DM2. As confirming it, after assessing the histocompatibility system, susceptible alleles were acknowledged as more frequent in both A+ subgroups than in A- subgroups. Resistance alleles, on the other hand, were found to be more frequent in β+ subgroups[67]. The inheritance of specific alleles may be considered as a marker of the pancreatic function evolution, determining the necessity of insulin and identifying candidates for immunomodulatory therapy.

A recent study[63] compared the diagnostic accuracy of the four classification items of ketosis-prone diabetes. The Aβ classification, with a 99.4% sensitivity, 95.9% specificity, 97.1% positive predictive value, 99.2% negative predictive value and a 0.972 area under the receiver operating characteristic curve (ROC), was statistically superior to the other three classifications for DM. Although this data are not definitive and further studies on diverse races are necessary for the confirmation of such findings, the magnitude of the differences with regard to other means of classification in current use suggests that it may be adopted as an effective, relatively simple and precise method for DM classification, complementing ADA and WHO's classifications.

To sum up, the Aβ classification, which allows for the presence or absence of antibodies (A+ or A-) and the β-cell function (β+ or β-), defines the presence of diabetes mellitus type 1A, 1B, 2A and 2B. These classifications maintain the already recognized DM1 subtypes, and extend them to other ethnicities. It also permits to identify type 2 patients that are initially insulin-dependent and later on may renounce its use, DM2B (A-β+) and also make notice the patients who have initially preserved pancreatic function, but may evolve to insulin dependence (A+β+), hence with periodic pancreatic function reviews needed.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by FIPE.

Study Association

This article is part of the thesis of master submitted by Jorge de Faria Maraschin, from *Universidade Federal do Rio Grande do Sul*.

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