Nitric Oxide 61 (2016) 1–9

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Nitric oxide levels in patients with diabetes mellitus: A systematic review and meta-analysis

Taís S. Assmann [a, b], Letícia A. Brondani [a], Ana P. Bouças [a], Jakeline Rheinheimer [a, b], Bianca M. de Souza [a, b], Luís H. Canani [a, b], Andrea C. Bauer [a], Daisy Crispim [a, b, *]

[a] Endocrine Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil
[b] Post-graduation Program in Medical Sciences: Endocrinology, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
30 August 2016
Accepted 23 September 2016
Available online 24 September 2016

Keywords:
Meta-analysis
Nitric oxide levels and diabetes mellitus

ABSTRACT

Background: Altered serum nitric oxide (NO) levels in patients with diabetes mellitus (DM) have been reported by different studies; however, results are still controversial. Until this date, no meta-analysis evaluated the association of NO levels with DM. Thus, this paper describes a meta-analysis conducted to evaluate if there is a relationship between NO levels and type 1 DM (T1DM) or type 2 DM (T2DM).
Methods: A literature search was done to identify all studies that investigated NO levels between T1DM or T2DM patients (cases) and non-diabetic subjects (controls). Measurement of nitrate and nitrite (NOx – the stable NO products) were used to estimate NO concentrations because they closely reflect NO bioavailability. Weighted mean differences (WMD) of NOx levels between case and control samples were calculated for T1DM and T2DM groups.
Results: Thirty studies were eligible for inclusion in the meta-analysis (8 in T1DM samples and 22 in T2DM samples). NOx levels were increased in European T1DM patients compared with controls [random effect model (REM) WMD = 8.55, 95% CI 2.88 – 14.21]. No other ethnicity was evaluated in T1DM studies. NOx levels were also increased in both European (REM WMD = 18.76, 95% CI 1.67 – 35.85) and Asian (REM WMD = 18.41, 95% CI 8.01 – 28.81) T2DM patients, but not in Latin American patients compared with controls.
Conclusions: This meta-analysis detected a significant increase in NOx levels in European T1DM patients as well as European and Asian T2DM patients. Further studies in other ethnicities are necessary to confirm these data.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1,2]. Depending on the intensity and duration of exposure to hyperglycemia, structural damage may occur in vascular endothelium and nervous tissue, leading to dysfunction, and even failure of different organs and tissues, characterizing the diabetic chronic complications [2]. These complications are divided into macrovascular- (coronary artery disease, peripheral vascular disease and stroke) and microvascular-complications (diabetic kidney disease, diabetic retinopathy and neuropathy), and are associated with high morbidity and mortality rates among diabetic patients [3].

Endothelial dysfunction appears to be a consistent finding in all diabetic patients. Indeed, there is a general agreement that chronic hyperglycemia and DM lead to impairment in nitric oxide (NO) production and activity [4,5]. NO is a short-lived gaseous free radical secreted by endothelium. Modifications in its bioavailability have been found to cause endothelial dysfunction, increasing susceptibility to hypertension, progression of atherosclerosis, hypercholesterolemia, thrombosis, stroke, DM and its chronic complications [6–8].

NO is synthetized as a byproduct of the conversion of its physiological precursor L-arginine to L-citrulline by a family of NO synthases (NOS). These enzymes comprise three distinct isoforms, encoded by three different genes: neuronal (nNOS codified by *NOS-*

* Corresponding author. Rua Ramiro Barcelos 2350, prédio 12; 4° andar, CEP 90035-003, Porto Alegre, RS, Brazil.
E-mail address: dcmoreira@hcpa.edu.br (D. Crispim).

http://dx.doi.org/10.1016/j.niox.2016.09.009
1089-8603/

1), inducible (iNOS/*NOS-2*), and endothelial (eNOS/*NOS-3*) forms [7,9]. Although, at baseline, the main source of plasma NO is related to eNOS, during several clinical conditions, such as inflammation, iNOS is activated [10]. NO acts as pleiotropic intracellular messenger, exerting a variety of biological actions under both physiological and pathological conditions [9]. While low levels of NO are beneficial for several physiological and cellular functions, keeping vascular tonus, coagulation and inflammation well balanced; high levels of NO may cause detrimental effects [11,12].

In diabetic patients, hyperglycemia stimulates the production of advanced glycation end products (AGEs), and enhances the polyol, protein kinase C (PKC) and hexosamine pathways, which may lead to oxidative stress [13,14]. Then, excessive reactive oxygen species (ROS), such as superoxide anion (O_2^-), react rapidly with NO radicals, forming the peroxynitrite anion, which is a toxic oxidant capable of damaging several biological molecules, leading to tissue injury [15–17].

NO is oxidized in vivo, producing the stable NO products nitrate and nitrite (NOx). Several findings suggest a causal relationship between NO and plasma levels of NOx and, then, NOx plasma measurements reflect NO bioavailability. Nevertheless, overproduction of peroxynitrite can deplete NO bioavailability [12,14].

Altered serum NO in type 1 DM (T1DM) or type 2 DM (T2DM) patients has been reported by several studies; however, results are controversial. Some studies reported increased NO levels in diabetic patients [18–22], whereas others reported the opposite [23–25]. Moreover, NO bioavailability was described as being decreased in animal models of obesity and DM [26–28]. Thus, to further investigate the association between serum/plasma NOx levels and T1DM or T2DM, we performed a systematic review and meta-analysis of the literature on the subject.

2. Methods

2.1. Search strategy and eligibility criteria

PubMed and Embase repositories were searched to identify all available case-control studies that compared serum or plasma NOx levels (mean ± SD) between DM patients (T1DM or T2DM) and non-diabetic subjects. The following medical subject headings (MeSH) were used for the search: ("type 1 diabetes mellitus" OR "type 2 diabetes mellitus" OR "diabetes mellitus" OR "diabetes complications") AND ("nitrate" OR "nitrite" OR "nitrosamine" OR "oxide nitric"). All articles identified were also searched manually to identify other relevant citations. Studies were excluded from analysis if they had insufficient data. If data were duplicated and had been published more than once, the most complete study was chosen.

The search was limited to human studies in English, Spanish or Portuguese language and was completed on 28 March 2016. This study was designed and described in accordance with current guidelines for execution of systematic reviews and meta-analyses [29,30].

2.2. Study selection and data extraction

Titles and abstracts of all identified eligible articles were screened by four investigators (T.S.A., L.A.B., J.R. and A.P.B.). For all potentially relevant articles, the full text was retrieved and reviewed independently, as in previous systematic reviews by our group [31–33]. Disagreements were resolved by discussion between them and when necessary another reviewer (D.C.) was consulted (less than 5% of articles).

Data from each included study were independently extracted by two investigators (T.S.A and L.A.B.) using a standardized extraction form, and consensus was sought in all extracted items. When consensus could not be reached, differences in data extraction were decided by referencing the original publication and by consulting a third reviewer (D.C.). The information extracted from each study was as follows: name of first author, publication year, ethnicity, sample size, age, gender, HbA1c levels, analytical method used, and NOx levels (mean ± SD) in diabetic patients and in non-diabetic subjects.

2.3. Quality assessment

To ascertain the validity of each included study, two investigators (T.S.A and L.A.B.) independently assessed the quality using the Newcastle-Ottawa Scale [34]. This scale contains eight items divided into three dimensions: selection, comparability, and exposure. For each item, a sequence of answer options is provided. A star scoring system is used to provide a semi-quantitative assessment of the paper quality, so that highest quality studies receive a maximum of one star for each item, with exception of the comparability item that can receive two stars. Hence, Newcastle-Ottawa score varies from zero to nine stars.

2.4. Statistical analyses for meta-analyses

Heterogeneity among studies was tested using a χ^2-based Cochran's *Q* statistic and inconsistency was assessed by the I^2 metric [35,36]. Heterogeneity was considered important if $P < 0.10$ for the *Q* statistic and $I^2 > 50\%$ for the I^2 statistic. Where significant heterogeneity was detected, the DerSimonian and Laird random effect model (REM) was used to calculate weighted mean differences (WMD) with 95% CI in NOx levels for each individual study and for the pooled effect; where heterogeneity was not significant, the fixed effect model (FEM) was used for this calculation, as previously described [31–33].

Meta-regression and sensitivity analyses were carried out to identify key studies with a substantial impact on inter-study heterogeneity. Factors included in meta-regressions were age, gender, and HbA1c. Sensitivity analyses were performed in order to estimate the influence of each individual study in the meta-analysis results. This was done by repeating meta-analyses omitting a different study each time. Moreover, meta-analyses were also performed after stratification of studies by ethnicity, type of blood sample analyzed, and method used for NOx measurement. Risk of publication bias was assessed using funnel plot graphics, analyzed both visually and with the Begg and Egger test [37]. The significance of the intercept was evaluated by *t*-test, with $P < 0.10$ considered indicative of significant publication bias. All statistical analyses were performed using Stata 11.0 software (StataCorp, College Station, TX, USA).

3. Results

3.1. Literature search and characteristics of eligible studies

Fig. 1 is a flow diagram illustrating the strategy used to identify and select studies for inclusion in this systematic review and meta-analysis. The search retrieved 2894 potentially relevant citations, and 2730 of them were excluded during review of titles and abstracts. One-hundred sixty-four articles appeared to be eligible at this point and had their full texts evaluated. After cautious analysis of the full texts, another 134 studies were excluded due to lack of information or ineligible study design. A total of 30 articles fulfilled the eligibility criteria and were included in the meta-analysis. Among them, 8 studies analyzed NOx levels in T1DM patients [20–22,38–42] and 22 studies comprised T2DM patients