© 2019 Journal of Pharmacy & Pharmacognosy Research, 7 (2), 103-115, 2019
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

The oxidative stress index in human population with arterial hypertension and diabetes mellitus

[El índice de estrés oxidativo en una población humana con hipertensión arterial y diabetes mellitus]

Alberto J. Núñez Sellés[1,2*], Wilfredo I. Mañon Rossi[1], Rodolfo Núñez Musa[2], Rafael Guillén Marmolejos[2], Gregorio Martínez-Sánchez[3]

[1]National Evangelic University (UNEV), Paseo de los Periodistas 54, Miraflores, CP 10203, Santo Domingo, Distrito Nacional, Dominican Republic.
[2]Dominican Institute of Clinical and Pharmacological Research (IDICF), Calle Primera, Bloque VI No. 12, Buenos Aires del Mirador, CP 11109, Santo Domingo, Distrito Nacional, Dominican Republic.
[3]Independent Consultor, Via Dalmazia 16A, 60126, Ancona, Italy.
*E-mail: nunez500412@gmail.com

Abstract

Context: A clinical study was conducted in a cohort of human subjects from 60 to 75 years of age in order to assess the correlation between disease diagnosis and oxidative stress (OS) damage in diabetes mellitus (DM) and arterial hypertension (AHT).

Aims: To provide a sound basis to design a clinical protocol for antioxidant adjuvant therapy for DM and AHT.

Methods: The values of the OS index were determined from the experimental values of biomarkers in erythrocyte lysates obtained from human blood samples including total antioxidant levels, specific biomarkers of oxidative damage, and antioxidant enzyme activities. Three study groups were formed: Group I: DM patients (110 subjects), group II: AHT patients (112 subjects); and Control group: Healthy volunteers (123 subjects). The data of all groups were analyzed using SPSS 9.0 software. A nonparametric Friedman test was used to compare several related subgroups, and changes within the groups and subgroups were tested using the Wilcoxon paired test. A Mann-Whitney U test was used to estimate significant differences ($p<0.05$) between the subgroups.

Results: Severe OS was observed in subgroup IIc (AHT III), moderate OS was observed in subgroups Ib (DM II) and IIb (AHT II), mild OS was observed in subgroup Ia (DM I), and no OS was observed in subgroup IIa (AHT I).

Conclusions: The results support the possible design of clinical trial protocols for adjuvant antioxidant therapy in order to increase the efficacy of standard therapies for AHT II and III and for DM II. Antioxidant therapies for DM I and AHT I are not recommended due to the presence of only mild OS or no OS, respectively.

Keywords: antioxidant therapy; diabetes mellitus; hypertension; oxidative stress; redox biomarkers.

Resumen

Contexto: Se realizó un estudio clínico en una cohorte de sujetos humanos entre 60 y 75 años de edad para determinar la correlación entre diagnóstico de la enfermedad y daño por estrés oxidativo (EO) en diabetes mellitus (DM) e hipertensión arterial (HTA).

Objetivos: Proporcionar una base sólida para el diseño de un protocolo clínico para la terapia adyuvante antioxidante en DM e HTA.

Métodos: Se determinaron los valores del índice de EO a partir de las determinaciones experimentales de niveles de antioxidantes totales, biomarcadores específicos del daño oxidativo y actividades de enzimas antioxidantes en lisados de eritrocitos obtenidos de muestras de sangre. Se conformaron tres grupos de estudio: Grupo I; pacientes DM (110 sujetos), grupo II; pacientes HTA (112 sujetos), y grupo III; voluntarios sanos (123 sujetos). Los datos de los grupos se analizaron con el programa SPSS 9.0. Se utilizó la prueba no paramétrica de Friedman para comparar entre subgrupos, y los cambios entre grupos se analizaron mediante la prueba de pares de Wilcoxon. Las diferencias significativas entre subgrupos ($p<0.05$) se estimaron con la prueba de Whitney U.

Resultados: Se observó EO severo en el subgrupo IIc (HTA III), EO moderado en los subgrupos Ib (DM II) y IIb (HTA II), EO leve en el subgrupo Ia (DM I), y no se observó EO en el subgrupo IIa (HTA I).

Conclusiones: Los resultados demuestran el posible diseño de protocolos de ensayos clínicos para incrementar la eficacia de las terapias estándar de la HTA II y III, y DM II, con el uso de terapias antioxidantes adyuvantes, las que no son recomendables para DM I y HTA I, debido a que el EO es leve o no existente, respectivamente.

Palabras Clave: biomarcadores redox; estrés oxidativo; diabetes mellitus; hipertensión arterial; terapia antioxidante.

ARTICLE INFO
Received: January 16, 2019.
Received in revised form: February 21, 2019.
Accepted: February 22, 2019.
Available Online: February 26, 2019.
Declaration of interests: The authors declare no conflict of interest.
Funding: This work was supported by the Ministry of Higher Education, Science and Technology (MESCYT), Dominican Republic, Project FONDOCYT 2012-2013-2A1-58, and by the National Evangelic University (UNEV), Santo Domingo, Dominican Republic, Project UNEV 2013/004.

INTRODUCTION

Alteration of the oxidative status in humans has been associated with several pathologies, including diabetes and cardiovascular diseases. However, the prognostic relevance of circulating oxidative stress (OS) biomarkers remains poorly understood. Clinical antioxidant therapy trials have failed to show beneficial effects of antioxidants in combating hypertension or diabetes. OS is not the only cause of hypertension and diabetes; however, it may amplify an increase in blood pressure and/or blood glucose levels in the presence of other stimulating factors. A lack of clinical evidence in the antioxidant therapy trials might be related to inadequate methods to measure the redox state of patients (Montezano et al., 2015). Most trials have selected antioxidants as an antihypertensive treatment due to availability of antioxidants and not because of their efficacy; OS biomarkers have not been properly selected and physiological targets have not been completely evaluated (Stephens et al., 2009). Nevertheless, the majority of reports have emphasized the introduction of antioxidant therapies for the management of OS induced by hypertension and DM as well as by their complications (Ahmad et al., 2017).

The selection of adequate OS biomarkers to assess the efficacy of an antioxidant therapy used to treat hypertension and/or DM is crucial for the success of a clinical trial. These biomarkers provide information about i) lipid peroxidation products, ii) protein modification products, iii) antioxidant enzyme activities, and iv) DNA modifications altering genes expression (Mañon et al., 2016). Much of the clinical data published on the use of antioxidants in hypertension and DM did not integrate all information about OS biomarkers (Frijhoff et al., 2015). Marrocco et al. (2017) have suggested that the bias of independently separated methods may be overcome by using those indexes of OS that include more than one marker relevant to the aim of the clinical study and to its clinical applications.

Recently, a procedure for determining the OSI that correlates to disease progression and OS status has been patented using a percentage scale that compares the OS parameters in patients with those in healthy volunteers of the same population, age, and sex (Núñez-Sellés et al., 2017). The results of the present study were obtained in a cohort formed to determine the OSI in human populations with AHT (degrees I, II, and III) and DM (types I, and II) compared to a control group of healthy volunteers with the goal of assessing the potential beneficial effect of the adjuvant antioxidant therapy using this integrated technique to measure OS in humans.

MATERIAL AND METHODS

Medical deontology

The clinical trial was designed as a cohort study with subjects aged from 60 to 75 years to prospectively assess the OS degree in hypertensive and/or diabetic patients treated in public health institutions in the Dominican Republic. Specifically, the study was conducted in the capital city, Santo Domingo, where this age group accounts for approximately 30% of the total population according to the most recent demographic census (National Office of Statistics, 2012). According to the last epidemiological study on DM and AHT in Dominican Republic (Society of Cardiology, Dominican Republic, 2012), 81,814 and 286,350 people were diagnosed with DM and AHT, respectively, in Santo Domingo Province. This trend may have remained at the time of the present trial.

The clinical protocol was approved by the Bioethics Commission, National Evangelic University (UNEV), and submitted to the National Commission of Health Bioethics (CONABIOS) of the Dominican Republic. Final approval of the cohort study was registered as document 008-2016 (Supplementary Information), and patients and healthy volunteers were recruited with their informed consent according to the Declaration of Helsinki from May-August 2016. Research assistants explained the purpose of the study to the patients and healthy volunteers, and the informed consent form was signed in the presence of a witness. All information about subject identities was main-

tained confidential, and the corresponding codes were used to identify the clinical records and samples.

Cohort study

The sample size that was required to obtain statistically significant results was determined using validated software (Faul et al., 2007) considering the incidence of DM and AHT in various age groups in the Santo Domingo population. Three groups and 10 subgroups were formed during the study to obtain a significance level (α) = 0.05, potency (1-β) = 0.2, standard deviation (σ) = 2, and detection difference (d) = 1. A total of 345 subjects were recruited.

Inclusion criteria were as follows: i) patients of both sexes from 60 to 75 years of age with a confirmed diagnosis of DM (types I and II) and/or AHT (degrees I, II, and III) from Santo Domingo Province, ii) patients with disease presence for at least 2 years or more iii) patients who were nonsmokers, iv) patients who did not regularly consume alcoholic drinks or stupefacient drugs, and v) patients who did not consume of antioxidants during the last 12 months. Exclusion criteria were as follows: i) other chronic degenerative diseases, and ii) participation in other clinical studies.

AHT patients were classified according to the *Clinical Practice Guides for Arterial Hypertension Treatment* (Mancia et al., 2007) as shown in Table 1. DM patients were classified according to *Diabetes Management Guidelines* (American Diabetes Association, 2016) as shown in Table 2.

Table 1. Classifications of AHT patients.

AHT degree	Systolic blood pressure	Diastolic blood pressure
I	140 - 159	90 - 99
II	160 - 179	100 - 109
III	≥180	≥110

AHT: Arterial hypertension.

Table 2. Classification of DM patients.

DM type	Control value
	Hyperglycemia acute symptoms
I	FPG > 200 mg/dL
II	FPG > 126 mg/dL

DM: Diabetes mellitus; FPG: Fasting plasma glucose.

Subjects were evaluated clinically by a physician, DM and AHT diagnoses were confirmed based on previous analysis, blood samples (100 µL) were collected and data were recorded in the Data Collection Logbook (DCL) in duplicate using a code. Lists with the personal data of included or excluded subjects were submitted by the physicians to the Principal Researcher.

Blood samples

Blood samples (100 µL) were collected by finger puncture with a sterilized lancet and transferred into an Eppendorf vial containing 2 mL saline. Samples were maintained at 8°C until transportation to the analytical laboratory within the next 24 h. Samples were centrifuged at 3,000 xg. The supernatant was discarded, and 2 mL of double-distilled water was added to the erythrocyte fraction to induce an osmotic shock and the consequence lysis. Fractions of erythrocyte (RBC) lysate (200 µL) were collected with a micropipette, transferred into Eppendorf vials (1 mL), coded, and maintained at -10°C in a vertical freezer until processing within the next 60 days.

Laboratory methods

Peroxidation potential (PP)

PP values were determined by the modified method of Ozdemirler et al. (1995). RBC lysates were incubated with copper II sulfate, 2 mM (JT Baker, USA) at 37°C for 24 h. Malondialdehyde was determined at 0 and 24 h (at 586 nm) after incubation. The difference in malondialdehyde values was assumed as the PP. Standard malondialdehyde was purchased from Sigma-Aldrich, Missouri, USA.

Total antioxidant status (TAS)

TAS values were determined based on the kinetics of formation of free radicals in the reaction with ABTS+) -2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation- (Sigma Aldrich, Missouri, USA) at 600 nm according to Ozcan (2004).

Trolox equivalent antioxidant capacity (TEAC)

TEAC values were determined by the modified method of Blois (1958). RBC lysates or Trolox were incubated with sodium acetate buffer, 40 mM, pH = 5.5, and 1 mL ethanol for 1 h at 37°C. Then, 1 mL of 100 µM 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added after incubation to an aliquot of the incubated sample and absorbance was determined at 517 nm in the aliquots with and without DPPH. Trolox and DPPH were purchased from Sigma-Aldrich, Missouri, USA; other reagents from JT Baker, USA.

Malondialdehyde (MDA)

MDA concentrations were determined according to Esterbauer and Cheeseman (1990). RBC lysates were analyzed using an LPO-586 kit (Calbiochem, La Jolla, CA, USA). The production of a stable chromophore after 40 min incubation at 45°C was measured at 586 nm. Freshly prepared solutions of MDA bis[dimethylacetal] were used as a standard and were assayed under identical conditions. MDA was purchased from Sigma-Aldrich, Missouri, USA.

Carbonyl groups (CG)

CG values were determined according to Oliver et al. (1987). RBC lysates were suspended in 1 mL hydrochloric acid (2 M) and 1 mL 2,4-dinitrophenylhydrazine (0.2 %) and incubated with stirring at 37°C for 1 h. Then, 1 mL 10 % trichloroacetic acid (TCA) was added and the precipitate was extracted with 10 mL ethanol:ethyl acetate (1:1). The solid was reprecipitated with TCA (10 %). Protein control sample (egg albumin) was dissolved in 1 mL guanidine hydrochloride in potassium phosphate buffer (20 mM, pH = 6.5). The sample and the control were centrifuged, and absorbance was determined at 370-375 nm. HCl, ethyl acetate, and ethanol were purchased from JT Baker, USA; DNPH, TCA, guanidine.HCl, and egg albumin were purchased from Sigma-Aldrich, Missouri, USA.

Sulfhydryl groups (SG)

SG values were determined according to Sedlak and Lindsay (1968). RBC lysates were dissolved in 1 mL potassium phosphate buffer (50 mM, pH = 7.5) and centrifuged. The supernatant was transferred into a vial containing Ellman's reagent and absorbance was determined at 600 nm. The same procedure was used in the case of the nonoxidized control protein sample (egg albumin). All reagents were purchased from JT Baker, USA, except egg albumin from Sigma-Aldrich, Missouri, USA.

Superoxide dismutase (SOD)

SOD activity was determined according to Mestro and McDonald (1986). RBC lysates were mixed with 2.8 mL buffer containing 100 µL 05 M Tris [tris(hydroxymethyl)aminomethane], pH = 8.2, in distilled water and 50 µL of EDTA solution (1 mM). Then, 50 µL pyrogallol solution (0.124 M) was added and absorbance values were determined at 420 nm after 10 s and then every 10 s for 1 min (six absorbance values). The same procedure was used in the case of a blank sample without blood. Absorbance differences between the sample and the blank were calculated and SOD activity was determined. All reagents were purchased from Sigma-Aldrich, Missouri, USA.

Catalase (CAT)

CAT activity was determined according to Haining and Legan (1972). RBC lysates were mixed with 0.9 mL potassium phosphate buffer (pH = 7) and 500 µL buffered hydrogen peroxide solution (0.05 M) in a quartz cell. Absorbance at 240 nm was determined at 10 and 70 s. Blank absorbance was determined using 1.5 mL buffer solution. Absorbance differences between the sample and the blank were calculated and CAT activity was determined. All reagents were purchased from Sigma-Aldrich, Missouri, USA.

Glutathione peroxidase (GPx)

GPx activity was determined according to Gil et al. (2003). GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized GSH is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. A decrease in absorbance at 340 nm was measured. GPx activity was expressed as U/g Hb. All reagents were purchased from Sigma-Aldrich, Missouri, USA.

Analytical results were recalculated into a scale of 100 units for comparison. Samples were analyzed in triplicate in every assay and the results were expressed as ($\bar{Y}$) ± SD. Solutions were prepared with bi-distilled water (Aqua MAXTM-Ultra 372, Seoul, Korea). Absorbance values were determined with a Genesis 10S spectrophotometer (Thermo Scientific, Massachusetts, USA) in 1 cm quartz cells; incubation was performed using disposable Petri dishes (Fisherbrand, Pittsburgh, USA) in a DNI-150 incubator (MRC, Israel); samples were centrifuged using a MiniSpin centrifuge (Eppendorf, Germany); blood samples were collected with AutoLancets (Palcolabs, Santa Cruz, CA, USA) in 0.5, 1, and 2 mL PVC vials (Eppendorf, Germany). Analytical results were recorded in the DCL in duplicate for subsequent data processing.

Data processing

All laboratory data were tabulated in independent tables for the Control group, group I (DM), and group II (AHT). The OS degree (OSD) was calculated in the healthy control group as a reference and the OSI values were calculated in the Groups I and II based on the OSD of the Control group as follows (Núñez-Sellés et al., 2017):

$$OSI\ (\%) = \left[\frac{n^1 Mi^A + n^2 Mi^B + n^3 Mi^C}{n^1 + n^2 + n^3}\right] G$$

where:

$$Mi^A = \frac{\sum T_A{}^{DM/AHT}}{\sum T_A{}^{HEALTHY}} \cdot 100$$

$$Mi^B = \frac{\sum T_B{}^{DM/AHT}}{\sum T_B{}^{HEALTHY}} \cdot 100$$

$$Mi^C = \frac{\sum T_C{}^{DM/AHT}}{\sum T_C{}^{HEALTHY}} \cdot 100$$

where n^1 = 3 (number of biomarkers for determination of total concentration of free radicals: peroxidation potential, total antioxidant status, and Trolox equivalent antioxidant capacity);

n^2 = 3 (number of specific biomarkers of cell/tissue oxidative damage: malondialdehyde, carbonyl groups, and sulfhydryl groups);

n^3 = 3 (number of specific biomarkers of endogenous antioxidant enzymes: Superoxide dismutase, catalase, and glutathione peroxidase activities);

G = sex (M or F) or age group (1: 60-64; 2: ≥ 65 years old).

All experimental data were processed from the DCL by two independent statisticians who generated duplicate tables. Data for all groups were analyzed using SPSS 9.0 software. The nonparametric Friedman test was used for several related subgroups and changes within the groups and subgroups were tested using Wilcoxon's paired test. The Mann-Whitney U test was used to estimate significant differences ($p<0.05$) between the subgroups. The results were expressed as the mean ± standard error of the mean (SEM) using a percentage scale (100 units). The oxidative damage degree (ODD) was determined as a percentage of the OSI determined in groups I and II as shown in Table 3.

Table 3. Classification of oxidative damage degree (ODD) according to the oxidative stress index (OSI).

OSI (%)	ODD
0	None
<30	Mild
30 - 60	Moderate
>60	Severe

RESULTS

The control group had similar age, ethnicity, and sex distributions as groups I and II with a mean age of 68.1 ± 6.2 years old, predominantly black and mixed ethnicities (41.4 ± 9.1 and 32.2 ± 8.2%, respectively) comprising approximately 70% of the population under study, and female as the major sex (59.7%). No significant differences ($p>0.05$) in the OSD were found between various ethnic groups (Tables 4 and 5). OSD in the control group was between 30 and 40 (100-unit scale) and was significantly higher in the subjects ≥65 years (Table 6).

Table 4. Mean age and gender data from the cohort population study.

Group	n (male)	n (female)	n (total)	Mean age
Control	**51**	**72**	**123**	**67.0 ± 6.0**
Group I (DM)	**41**	**69**	**110**	**67.9 ± 4.0**
Subgroup Ia (DM type I)	13	24	37	66.5 ± 3.9
Subgroup Ib (DM type II)	28	45	73	68.7 ± 4.0
Group II (AHT)	**47**	**65**	**112**	**69.4 ± 6.6**
Subgroup IIa (AHT degree I)	20	30	50	67.9 ± 5.0
Subgroup IIb (AHT degree II)	15	21	36	70.4 ± 5.8
Subgroup IIc (AHT degree III)	12	14	26	71.0 ± 5.5
TOTAL	**139**	**206**	**355**	**68.1 ± 6.2**

DM: Diabetes mellitus, AHT: Arterial hypertension. No significant differences ($p>0.05$) were found between age groups.

Table 5. Ethnic composition of the cohort population study.

Group	n (white)	%	n (black)	%	n (mixed)	%	n (asian)	%	Total
Control	**23**	**18.7**	**61**	**49.6**	**37**	**30.1**	**2**	**1.6**	**123**
Group I (DM)	**32**	**29.1**	**34**	**30.9**	**44**	**40.0**	**0**	**0**	**110**
Subgroup Ia (DM type I)	10	27.0	12	32.4	15	40.5	0	0	37
Subgroup Ib (DM type II)	22	30.1	22	30.1	29	39.7	0	0	73
Group II (AHT)	**33**	**29.5**	**48**	**42.9**	**30**	**26.8**	**1**	**0.8**	**112**
Subgroup IIa (AHT degree I)	12	24.0	22	44.0	16	32.0	0	0	50
Subgroup IIb (AHT degree II)	14	38.9	17	47.2	4	11.1	1	2.8	36
Subgroup IIc (AHT degree III)	7	26.9	9	34.6	10	38.5	0	0	26
TOTAL	**88**	**25.5**	**143**	**41.4**	**111**	**32.2**	**3**	**0.9**	**345**

DM: Diabetes mellitus, AHT: Arterial hypertension. No significant differences ($p>0.05$) were found between ethnic groups.

Table 6. Determination of the oxidative stress degree (OSD) in Control group.

Gender	Age range (years old)	Term M_i^A	Term M_i^B	Term M_i^C	OSD ± SD ($p<0.05$)
Male	60 - 64	11.0 ± 2.3	10.8 ± 1.3	9.0 ± 1.3	30.7 ± 3.3
	≥65	14.0 ± 2.4	14.7 ± 1.9	11.5 ± 1.6	40.2 ± 5.7^{ab}
Female	60 - 64	10.7 ± 1.6	10.8 ± 1.9	9.2 ± 1.4	30.7 ± 5.4
	≥65	13.3 ± 1.2	14.6 ± 2.0	12.1 ± 1.5	40.0 ± 4.8^{a}

Different letters mean statistical difference ($p<0.05$) between the same series. $M_i^A = A_1$ (PP) + A_2 (TAS) + A_3 (TEAC). $M_i^B = B_1$ (MDA) + B_2 (carbonyl groups) + B_3 (sulfhydryl groups). $M_i^C = C_1$ (SOD) + C_2 (CAT) + C_3 (GPx). PP: Peroxidation potential, TAS: Total antioxidant status, TEAC: Trolox equivalent antioxidant capacity, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase.

The OSI values in subgroup Ia (DM I) were not significantly different from the OSI values in the control group, except for the female patients ≥65 years of age, where the OSI value was significantly lower. However, the OSI values in Subgroup Ib (DM II) were significantly higher in both sexes than those in the Control group and subgroup Ia, varying from 40 to 50 % (Table 7).

The results in subgroup IIa (AHT I) showed negative values of OSI (Table 8). The OSI values in Subgroups IIb and IIc were significantly higher than those in the Control group and subgroup Ia, which ranged from 47 to 67 % and from 65 to 71 %, respectively. The highest significant difference was found in the female group ≥65 years of age.

The estimation of ODD for groups I and II is shown in Table 9. Of note, no ODD was detected in AHT I (subgroup IIa), mild ODD was detected in DM I (subgroup Ia), moderate ODD was detected in AHT II (subgroup IIb) and DM II (subgroup Ib) and severe ODD was detected in AHT III (subgroup IIc).

DISCUSSION

OS biomarkers in RBC lysates have been considered a better measure of the OS of subjects than those in the serum or plasma (Peruzzi et al., 2019). The oxidation of biomolecules has been reported to be more accurately detected in RBC lysates than in the serum or plasma by several authors (Zilmer et al., 2005; Yoshida et al., 2007). Therefore, we decided to perform OS biomarker tests in RBC lysates. We also decided to exclude smokers from the study since their inclusion could introduce bias to the characterization of the oxidative stress induced by the pathology. Smoking is a known factor that stimulates oxidative stress.

An OS diagnosis based on the percentage scale considers the OS status of a specific age population with diagnosed DM and/or AHT against the OS status of an age-matched control group without DM and/or AHT, which means that OSI would detect OS related to the disease alone. The analytical results of the present study cannot be

Table 7. Determination of oxidative stress index in group I (diabetes mellitus).

Gender	Age range (years old)	Term M_i^A	Term M_i^B	Term M_i^C	OSI ± SD ($p<0.05$)
Diabetes mellitus, type I (subgroup Ia)					
Male	60 - 64 y	17.4 ± 2.6	17.6 ± 1.9	11.2 ± 1.0	33.0 ± 6.8
	≥65 y	24.4 ± 2.8^a	24.8 ± 1.8^a	17.0 ± 1.4^a	29.0 ± 8.6
Female	60 - 64 y	20.9 ± 2.6	22.0 ± 1.8	14.5 ± 1.4	30.3 ± 7.6
	≥65 y	26.9 ± 2.5^{ab}	28.1 ± 1.5^{ab}	20.9 ± 1.5^{ab}	26.1 ± 8.8^a
Diabetes mellitus, type II (subgroup Ib)					
Male	60 - 64 y	24.4 ± 1.8	24.8 ± 1.4	17.0 ± 2.1	53.4 ± 3.5^b
	≥65 y	26.9 ± 2.7	28.1 ± 2.7	20.9 ± 3.8	46.1 ± 7.2^c
Female	60 - 64 y	23.8 ± 1.2	24.3 ± 1.5	16.9 ± 2.3	52.4 ± 3.6^c
	≥65 y	25.7 ± 1.9	27.1 ± 2.2	20.1 ± 3.7	43.9 ± 6.1^{abc}

Different letters mean statistical difference ($p<0.05$) between the same series. OSI: Oxidative stress index. $M_i^A = A_1$ (PP) + A_2 (TAS) + A_3 (TEAC). $M_i^B = B_1$ (MDA) + B_2 (carbonyl groups) + B_3 (sulfhydryl groups). $M_i^C = C_1$ (SOD) + C_2 (CAT) + C_3 (GPx). PP: Peroxidation potential, TAS: Total antioxidant status, TEAC: Trolox equivalent antioxidant capacity, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase.

Table 8. Determination of oxidative stress index in group II (arterial hypertension).

Gender	Age range (years old)	Term M_i^A	Term M_i^B	Term M_i^C	OSI ± SD ($p < 0.05$)
Arterial hypertension, degree I (subgroup IIa)					
Male	60 - 64 y	9.7 ± 1.0	10.3 ± 1.3	8.7 ± 1.4	-5.8 ± 12.4
	≥65 y	12.9 ± 0.8[a]	12.6 ± 1.3	11.4 ± 1.8	-9.7 ± 12.3
Female	60 - 64 y	9.5 ± 0.9	10.3 ± 1.2	8.9 ± 1.5	-6.2 ± 12.8
	≥65 y	14.3 ± 1.0[ab]	14.0 ± 1.4[a]	13.3 ± 1.4[a]	6.4 ± 7.8
Arterial hypertension, degree II (subgroup IIb)					
Male	60 -64 y	25.7 ± 2.7	26.7 ± 2.6	19.2 ± 3.1	56.6 ± 5.3 [abcd]
	≥65 y	29.8 ± 2.4	31.1 ± 2.1	23.1 ± 3.2	51.8 ± 4.5 [abcd]
Female	60 - 64 y	25.4 ± 2.3	26.6 ± 1.7	18.5 ± 2.9	56.0 ± 4.5 [abcd]
	≥65 y	38.7 ± 1.9[a]	42.5 ± 2.1[a]	36.3 ± 3.1[a]	65.9 ± 2.0 [abcd]
Arterial hypertension, degree III (subgroup IIc)					
Male	60 - 64 y	31.7 ± 2.3	35.3 ± 1.9	29.0 ± 2.7	67.9 ± 2.3 [abcd]
	≥65 y	39.3 ± 1.8[a]	43.6 ± 1.2	37.5 ± 1.2[a]	66.5 ± 1.2 [abcd]
Female	60 - 64 y	31.6 ± 2.6	35.1 ± 1.8	28.3 ± 2.0	67.5 ± 2.4 [abcd]
	≥65 y	40.6 ± 2.4[a]	46.0 ± 2.4[ab]	43.1 ± 2.6[ab]	69.1 ± 1.8 [abcde]

Different letters mean statistical difference ($p<0.05$) between the same series. OSI: Oxidative stress index. $M_i^A = A_1$ (PP) + A_2 (TAS) + A_3 (TEAC). $M_i^B = B_1$ (MDA) + B_2 (carbonyl groups) + B_3 (sulfhydryl groups). $M_i^C = C_1$ (SOD) + C_2 (CAT) + C_3 (GPx). PP: Peroxidation potential, TAS: Total antioxidant status, TEAC: Trolox equivalent antioxidant capacity, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase.

Table 9. Determination of the oxidative damage degree in groups I and II according to the oxidative stress index.

Gender	Age range (years old)	OSI				
		Subgroup Ia (DM type I)	Subgroup Ib (DM type II)	Subgroup IIa (AHT degree I)	Subgroup IIb (AHT degree II)	Subgroup IIc (AHT degree III)
Male	60 - 64 y	33.0 ± 6.8	53.4 ± 3.5[b]	-5.8 ± 12.4	56.6 ± 5.3 [abcd]	67.9 ± 2.3 [abcd]
	≥65 y	29.0 ± 8.6	46.1 ± 7.2[c]	-9.7 ± 12.3	51.8 ± 4.5 [abcd]	66.5 ± 1.2 [abcd]
	ODD	**MILD**	**MODERATE**	**NONE**	**MODERATE**	**SEVERE**
Female	60 -64 y	30.3 ± 7.6	52.4 ± 3.6[c]	-6.2 ± 12.8	56.0 ± 4.5 [abcd]	67.5 ± 2.4 [abcd]
	≥65 y	26.1 ± 8.8[a]	43.9 ± 6.1[abc]	6.4 ± 7.8	65.9 ± 2.0 [abcd]	69.1 ± 1.8 [abcde]
	ODD	**MILD**	**MODERATE**	**NONE**	**SEVERE**	**SEVERE**

OSI: Oxidative stress index; ODD: Oxidative damage degree; DM: Diabetes mellitus; AHT: Arterial hypertension.

extrapolated to other age populations or geographical origins with unknown baseline data for the OS biomarkers used.

The aim to demonstrate how a single measure of any type of OS biomarker reflects the responses of the body to external and/or internal factors and implies a useful tool to demonstrate the role of or relationship between these biomarkers and the pathophysiology of a disease. Measuring the baseline values in naive situations in the case of a single test of biomarkers has resulted in a valuable information in some cases (Lee et al., 2007).

Control group

The high OSD values in the control group between 30 and 40 (100 unit scale) in both sexes can be explained, at least partially, by the age range of the subjects. The role of reactive oxygen species (ROS) in the aging process is widely acknowledged (Sas et al., 2018). These epidemiological results are the first to examine oxidative damage in a healthy Dominican population over 60 years of age and have included nine biomarkers characterizing the overproduction of free radicals (term A), oxidative damage of biomolecules (term B), and activities of the enzymes related to the endogenous antioxidant defense mechanism (term C). Block et al. (2002) have emphasized the importance of these types of studies to correlate the associations between OS and disease progression under standard treatment for a set of biomarkers; these studies may lead to the understanding of redox balance in human health. Specifically, these results were used to determine the OSI in DM and AHT patients by comparing their OSD values to that of the control group.

Group I (diabetes mellitus)

It is well known that hyperglycemia-induced alterations in glucose metabolism, lipid peroxidation, advanced glycation, glutamate toxicity, and OS enhance the apoptosis of the retinal capillary endothelial cells and increase the expression of VEGF and VEGFR2, which are crucial factors in the development of diabetic microangiopathy (Mondal et al., 2018). Although significant differences ($p<0.05$) were found for the independent terms of the OSI equation in subgroup Ia, the standard deviation was considerably higher in the case of OSI, which supports the hypotheses of other researchers that an index of OS biomarkers is better suited for the detection of OSD than a single biomarker (Frijhoff et al., 2015; Marroco et al., 2017). The mild ODD values observed in DM I (subgroup Ia) lead to the hypothesis that antioxidant therapy is not recommended in this type of DM. However, the moderate ODD values in DM II indicated that adjuvant antioxidant therapy with the standard DM II treatment may be more effective in to reduce OS. Therefore, disease diagnosis (type I or II) DM should be taken into account when considering clinical intervention with antioxidants. Lodovicia et al. (2008) have studied the oxidative DNA damage and plasma antioxidant capacity in DM II patients and demonstrated that plasma antioxidant level was significantly lower ($p<0.05$) than the healthy control group, whereas Escobar Aedo et al. (2011) did not detect significant differences in a clinical antioxidant study in DM I. Folli et al. (2011) recently reviewed the role of oxidative stress in the pathogenesis of DM II and concluded that the combination of adjuvant antioxidant therapies with the standard DM treatment in type II DM may improve survival and reduce diabetic comorbidities; the studies of the combination therapy indicate that hyperglycemia treatment alone does not improve survival in the patients with DM II.

Group II (arterial hypertension)

Hypertension is one of the major risk factors for cardiovascular diseases and is considered a leading cause of mortality and morbidity. Hypertension affects more than 600 million people and it has been estimated that 29 % of the world population will be suffering from hypertension by 2025. It has been indicated by experimental evidence that ROS play an important role in the pathophysiology of hypertension (Sinha and Dabla, 2015).

The results from subgroup IIa (AHT I) showed that OS was not present in these patients; moreover, the majority of the OS levels were lower than those in the healthy control group, indicating that antihypertensive therapy exerted a considerable antioxidant effect (Table 8). The majority of the patients in this subgroup were treated with the combination of an angiotensin II receptor blocker and a statin, both of which have been reported to have antioxidant effects (Yao et al., 2007; Profumo et al., 2014). Simic et al. (2006) reported that in mild hypertension, endogenous antioxidant enzyme activities are not decreased and therefore, antioxidant treatment in early stages of human hypertension is not critical.

Physiologically, ROS regulate vascular function through redox-sensitive signaling pathways. In

hypertension, OS promotes endothelial dysfunction, vascular remodeling, and inflammation, leading to vascular damage. Although experimental evidence indicates a causative role for OS in hypertension, the human data are less convincing. This may be related, in part, to suboptimal methods used to assess the redox state (Montezano et al., 2015). The results of subgroups IIb and IIc indicated the presence of moderate and severe ODD, respectively (Table 9), signifying that an increase in OSI may lead to higher degrees of hypertension. The question whether antioxidant therapy would be recommended in these subgroups remains unanswered. Most clinical trials on the use of the antioxidant vitamins C, E, and A (β-carotene) or their combinations to lower blood pressure have failed (Heart Protection Study Collaborative Group, 2002), which has limited support for the use of antioxidants as adjuvants as antihypertensive therapies. Nevertheless, the use of antioxidant cocktails, considering the different targets of the antioxidant therapy, has not been reported. At present, it is not clear what mechanisms should be targeted and what types of compounds should be used in order to increase the efficacy of standard antihypertensive treatment with antioxidants (Münzel et al., 2010).

A number of studies have reported the beneficial effect of dietary antioxidants in hypertension and cardiovascular risk; however, the lack of efficacy in lowering the blood pressure in the cases of AHT has been highlighted in other scenarios (Yusuf et al., 2000). Nonetheless, other negative factors associated with chronic diseases are involved in their pathogeneses, and OS plays a major role; hence, AHT and DM, in particular, may benefit from balanced diets containing antioxidant components (Poprac et al., 2017). It should be noted that more than one (three or four) antioxidant components are expected to be combined in a formulation to provide a real benefit in the adjuvant treatment of AHT and DM (Wu et al., 2014). This may be because balanced diets enriched with a wide variety of fruits are advantageous in improving the levels of blood sugar and blood pressure in certain cases; thus, complimentary therapy against the endothelial dysfunctions associated with AHT II and III and DM II may interfere with the OS–mediated signaling pathways and is a safe and promising recommendation.

The present study heads its conclusions to bet for a correct application of effective antioxidant therapy may target the pathophysiogical changes involved in the development of the disease and may prevent the complications or lower their occurrence and severity. The favorable effects of antioxidants given as a supplement in DM, even if specific antioxidant needs have not been determined, may significantly improve prognoses and were reported to prevent the complications (Testa et al., 2016).

Thus, complications of DM II and/or AHT II-III and OS related to these diseases may be prevented or ameliorated by the opportune application of antioxidant adjuvant therapy and may benefit by the selection of appropriate and specific compounds.

CONCLUSIONS

Our experimental evidence suggests that an appropriate OS diagnosis can optimize an intervention with an antioxidant cocktail. OSI determination in DM II and AHT II and III within a well-designed clinical protocol will provide the necessary support to optimize the use of antioxidant therapies in these diseases to increase efficacy and to decrease comorbidities in combination within the standard therapies currently used to treat these diseases. Currently, despite considerable controversy, increasing use of antioxidants in combination with the standardized antihypertensive or antidiabetic therapies appears to be the most effective means to improve the prognosis of DM II and AHT II and III due to control over the molecular mechanisms involved in the regulation of vascular function, metabolic balance and redox states by proper management with specific antioxidants (Sorriento et al., 2018). The application of antioxidant therapies may be useful to combat complications or deteriorations due to physiological imbalances in DM and AHT.

The results of the present trial support secondary prevention and indicate that the use of antiox-

idant therapies in DM I and AHT I should not be recommended in patients who have demonstrated mild antioxidant responses to their current treatment strategies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Higher Education, Science and Technology (MESCYT), Dominican Republic, Project FONDOCYT 2012-2013-2A1-58, and by the National Evangelic University (UNEV), Santo Domingo, Dominican Republic, Project UNEV 2013/004. The authors thank R. Silverio, D.A. Terrero, and M. Beras for their assistance in recruiting patients in the hospitals. D. Jackson, L. Kelly, J.M. Jimenez, and L. Perez for their assistance in recruiting healthy volunteers. M. Butler and P. Santana for the logistic support by the Faculty of Health Sciences, UNEV.

SUPPLEMENTARY INFORMATION

Clinical Trial Approval by the National Regulatory Commission. Protocol No. 008-2016. (www.conabios.gob.do).

REFERENCES

Ahmad KA, Yuan Yuan D, Nawaz W, Ze H, Zhuo CX, Talal B, Qilong D (2017) Antioxidant therapy for management of oxidative stress induced hypertension. Free Radic Res 51: 428–438.

American Diabetes Association (2016) Standards of medical care in diabetes-2016. Diabetes Care 39: S1–S106.

Block G, Dietrich M, Norkus EP, Morrow JD, Hudes M, Caan B, Packer L (2002) Factors associated with oxidative stress in human populations. Am J Epidemiol 156:274–285.

Blois M (1958) Antioxidant determinations by the use of a stable free radical. Nature 181: 1199–2000.

Escobar Aedo AL, Pérez Ortega Y, Vera González M, Peñaranda Guzmán O, Álvarez León A (2011) Effect of Vimang supplementation on oxidative stress markers in young patients with diabetes mellitus type 1. Rev Cub Endocrinol 22: 103–117.

Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 186: 407–421.

Faul F, Erdfelder E, Lang AG, Buchner A (2007) GPower 3: A flexible statistical power analysis for the social, behavioral and biomedical sciences. Behav Res Meth 39: 175–191.

Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, Muscogiuri G (2011) The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro-and macrovascular complications: Avenues for a mechanistic-based therapeutic approach. Curr Diab Rev 7: 313–324.

Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, Knight AR, Taylor EL, Oettrich J, Ruskovska T, Gasparovic AC, Cuadrado A, Weber D, Poulsen HE, Grune T, Schmidt HHW, Ghezzi P (2015) Clinical relevance of biomarkers of oxidative stress. Antioxid Redox Signal 23: 1144–1170.

Gil L, Martínez G, González I, Tarinas A, Álvarez A, Giuliani A, Molina R, Tápanes R, Pérez J, León OS (2003) Contribution to characterization of oxidative stress in HIV/AIDS patients. Pharmacol Res 47: 217–224.

Haining JL, Legan JS (1972) Improved assay for catalase based upon steady-state substrate concentration. Anal Biochem 45: 469–479.

Heart Protection Study Collaborative Group (2002) MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: A randomised placebo-controlled trial. Lancet 360: 23–33.

Lee SA, Kallianpur A, Xiang YB, Wen W, Cai Q, Liu Q, Shu XO (2007) Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. Cancer Epidemiol Biomark Prev 16: 2464–2470.

Lodovicia M, Giovannellia L, Pitozzia V, Bigaglia E, Bardinib G, Rotellab CM (2008) Oxidative DNA damage and plasma antioxidant capacity in type 2 diabetic patients with good and poor glycaemic control. Mutat Res 638: 98–102.

Mancia G, Backer GD, Dominiczak A, Cifkova R, Fagard R., Germano G, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Struijker Boudier HAJ, Zanchetti A (2007) Guías de práctica clínica para el tratamiento de la hipertensión arterial 2007. Rev Esp Cardiol 60(9): 968.e1-e94.

Mañon Rossi W, Garrido G, Nuñez Selles AJ (2016) Biomarkers of oxidative stress in antioxidant therapy. J Pharm Pharmacogn Res 4: 62–83.

Marrocco I, Altieri F, Peluso I (2017) Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxid Med Cell Long 2017: 6501046.

Mestro RF, McDonald W (1986) Oxidative enzymes in tissue homogenates in Greenwald RA (ed) CRC Handbook for Oxygen Radical Research. CRC Press, Boca Raton FL. p. 291–296.

Mondal LK, Bhaduri G, Bhattacharya B (2018) Biochemical scenario behind initiation of diabetic retinopathy in type 2 diabetes mellitus. Indian J Ophthalmol 66:535–540.

Montezano AC, Dulak-Lis M, Tsiropoulou S, Harvey A, Briones AM, Touyz RM (2015) Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. Can J Cardiol 31: 631–641.

Münzel T, Gori T, Bruno RM, Taddei S (2010) Is oxidative stress a therapeutic target in cardiovascular disease? Eur Heart J 31: 2741–2748.

National Office of Statistics (2012) Dominican Republic: Vital Statistics 2010. IX National Census of Population & Housing. Santo Domingo, Dominican Republic.

Nuñez Sellés AJ, Martínez G, Mañón Rossi W (2017) Method for determining oxidative stress index in patients with diabetes mellitus and arterial hypertension. Oficina Nacional de la Propiedad Industrial (ONAPI), República Dominicana. 2017; P2017–54, March 14.

Oliver CN, Ahn B, Moerman EJ, Goldstein S, Stadtman ER (1987) Age related changes in oxidised protein. J Biol Chem 262: 5488–5491.

Ozcan E (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS cation. Clin Biochem 37: 277–285.

Ozdemirler G, Mehmetcik G, Oztezcan S, Toker G, Sivas A, Uysal M (1995) Peroxidation potential and antioxidant activity of serum in patients with diabetes mellitus and myocardial infarction. Horm Metab Res 27: 194–196.

Peruzzi C, Nascimento S, Gauer B, Nardi J, Sauer E, Göethel G, Cestonaro L, Fão N, Cattani S, Paim C, Souza J, Gnoatto D, Garcia SC (2019) Inflammatory and oxidative stress biomarkers at protein and molecular levels in workers occupationally exposed to crystalline silica. Environ Sci Pollut Res Int 26(2): 1394–1405.

Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M (2017) Targeting free radicals in oxidative stress-related human diseases. Tr Pharmacol Sci 38: 592–607.

Profumo E, Buttari B, Saso L, Rigano R (2014) Pleiotropic effects of statins in atherosclerotic disease: focus on the antioxidant activity of atorvastatin. Curr Top Med Chem 14: 2542–2551.

Sas K, Szabó E, Vécsei L (2018) Mitochondria, oxidative stress and the kynurenine system, with a focus on ageing and neuroprotection. Molecules 23: pii: E191.

Sedlak J, Lindsay RH (1968) Estimation of total protein bond and non-protein sulfhydryl group with Ellman's reagent. Anal Biochem 25: 192–205.

Simic DV, Mimic-Oka J, Pljesa-Ercegovac M, Savic-Radojevic A, Opacic M, Matic D, Simic T (2006) Byproducts of oxidative protein damage and antioxidant enzyme activities in plasma of patients with different degrees of essential hypertension. J Hum Hypertens 20: 149–155.

Sinha N, Dabla PK (2015) Oxidative stress and antioxidants in hypertension-a current review. Curr Hypertens Rev 11: 132–142.

Society of Cardiology, Dominican Republic (2012) Study of Cardiovascular Risk Factors and Metabolic Syndrome II. (EFRICARD II -in Spanish-). Santo Domingo, Dominican Republic.

Sorriento D, De Luca N, Trimarco B, Iaccarino G (2018) The antioxidant therapy: New insights in the treatment of hypertension. Front Physiol 9: 528.

Stephens JW, Khanolkar MP, Bain SC (2009) The biological relevance and measurement of plasma markers of oxidative stress in diabetes and cardiovascular disease. Atheroscl 202: 321–329.

Testa R, Bonfigli AR, Genovese S, De Nigris V, Ceriello A (2016) The possible role of flavonoids in the prevention of diabetic complications. Nutrients 8: 310.

Wu Y, Tang L, Chen B (2014) Oxidative stress: Implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives. Oxid Med Cell Long 2014: 752387.

Yao EH, Fukuda N, Matsumoto T, Kobayashi N, Katakawa M, Yamamoto C, Tsunemi A, Suzuki R, Ueno T, Matsumoto K (2007) Losartan improves the impaired function of endothelial progenitor cells in hypertension via an antioxidant effect. Hypertens Res 30: 1119–1128.

Yoshida Y, Saito Y, Hayakawa M, Habuchi Y, Imai Y, Sawai Y, Niki E (2007) Levels of lipid peroxidation in human plasma and erythrocytes: Comparison between fatty acids and cholesterol. Lipids 42: 439–449.

Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G (2000) Effects of an angiotensin-converting-enzyme, ramipril, on cardiovascular events in high-risk patients. N Engl J Med 342: 145–153.

Zilmer M, Soomets U, Rehema A, Langel Ü (2005) The glutathione system as an attractive therapeutic target. Drug Design Rev 22: 121–127.

AUTHOR CONTRIBUTION:

Contribution	Núñez Sellés AJ	Mañon Rossi WI	Núñez Musa R	Guillén Marmolejos R	Martínez-Sánchez G
Concepts or ideas	x				x
Design	x				
Definition of intellectual content	x	x			x
Literature search	x		x		x
Experimental studies	x	x	x	x	x
Clinical trials	x	x	x	x	
Data acquisition		x	x	x	x
Data analysis	x		x		x
Statistical analysis	x				
Manuscript preparation	x	x	x	x	x
Manuscript editing	x		x		x
Manuscript review	x	x	x	x	x

Citation Format: Núñez Sellés AJ, Mañon Rossi WI, Núñez Musa R, Guillén Marmolejos R, Martínez-Sánchez **G** (2019) The oxidative stress index in human population with arterial hypertension and diabetes mellitus. J Pharm Pharmacogn Res 7(2): 103–115.