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Assessment of plasma catecholamines in patients with dysmetabolic iron overload syndrome

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Abstract
Background: Dysmetabolic iron overload syndrome (DIOS) is characterized by hyperferritinemia and normal transferrin saturation level with components of metabolic syndrome (MS). Among cases of MS, we determined those with DIOS and their characterizations, then we evaluated the association between plasma catecholamines status and hypertension in DIOS.
Methods: We compared 101 hypertensive patients with 50 healthy participants (control group). Iron (iron, transferrin, and ferritin), insulin, and plasma catecholamine (adrenaline, noradrenaline, and dopamine), profiles were measured for both groups. Homeostasis model assessment of insulin resistance index and transferrin saturation were also calculated.
Results: Out of 101 hypertensive patients, 64 were diagnosed with MS, and 6 of the latter met the DIOS diagnostic criteria. Significantly, DIOS patients were older and had lower body mass index (BMI) compared with hypertensive non-DIOS patients with *p*-values of (0.026), and (0.033), respectively. Adrenaline, noradrenaline, and dopamine levels did not differ significantly between DIOS and non-DIOS patients.
Conclusions: Of the MS patients, 9.3% were diagnosed with DIOS. Accordingly, complete iron profiling should be performed routinely in the cases of MS for early diagnosis of DIOS, to prevent future complications. Further studies are required to test the hypothesis linking older age and lower BMI with the pathogenesis of DIOS.

Keywords: BMI; Catecholamines, DIOS; Hyperferritinemia, Hypertension; Metabolic syndrome

Highlights:
- Among metabolic syndrome cases, 9% had Dysmetabolic iron overload syndrome (DIOS).
- DIOS cases were older and had lower BMI compared with hypertensive non-DIOS cases.
- Routine full iron profile is advisable for metabolic syndrome cases to detect DIOS.

Introduction

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) has outlined the definition of metabolic syndrome (MS) as the presence of any three out of five abnormal criteria. These include, abdominal obesity, elevated serum triglycerides (TG), decreased serum high-density lipoprotein cholesterol (HDL-c), high blood pressure (BP), and increased fasting plasma glucose (FG) (Alberti et al., 2009). Around 15–20% of MS cases manifest associated dysmetabolic iron overload syndrome (DIOS), which is a condition characterized by visceral and hepatic iron accumulation (Dongiovanni et al., 2011; Lainé et al., 2017; Ruivard et al., 2009). DIOS-related reports are scarce. It is important to distinguish DIOS-associated MS from other cases of MS, as iron accumulation carries the risk of increased cardiovascular complications and hepatocellular carcinoma (Kew, 2014).

Initially, DIOS was diagnosed using the criteria described by Mendler et al. (1999), which required the presence of one or more MS components, in association with hepatic iron overload as assessed by liver biopsy or magnetic resonance imaging.

However, such criteria were difficult to apply to cases not indicative of liver biopsy.

DIOS can be characterized by increased serum ferritin in the absence of any other cause of iron overload or inflammation and in the presence of normal transferrin saturation (TSAT) of 25–45%. Notably, dysmetabolic hyperferritinemia and DIOS might be considered as two sides of the same problem (Rametta et al., 2020).

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Pathogenesis of DIOS was suggested to be secondary to altered regulation of iron transport associated with insulin resistance (IR) and subclinical inflammation, often in the presence of predisposing genetic factors (Dongiovanni et al., 2011). Therefore, iron overload may directly or indirectly, through metabolic IR or inflammatory/autoimmune mechanisms, be linked to sympathetic nervous system stimulation, explaining hypertension seen among patients with DIOS (Seravalle et al., 2015). Sympathetic nervous system assessment by assaying catecholamines has been used (Goldstein, 2010).

Herein, we aimed to test the hypothesis linking catecholamine excess with the development of hypertension in DIOS patients. We also aimed to identify the prevalence of DIOS among patients with MS, as well as to characterize the clinical and laboratory features of DIOS. We recruited a group of hypertensive patients (with or without MS) and compared them with apparently healthy normotensive controls. After thorough assessments to identify MS cases, we carried out laboratory tests to further identify the prevalence of DIOS among MS patients. Catecholamines were then assayed as an indicator of the sympathetic drive for all participants (cases and controls) using radioimmunoassay (RIA).

Materials and methods

Subjects

A total of 101 hypertensive adult patients were enrolled from the outpatient clinics of internal medicine, Kasr Al Ainy, School of Medicine, Cairo University, Egypt, with 50 healthy participants (of both sexes, average age of 44 years) serving as the control group.

Inclusion criteria

To make the diagnosis of MS, we required three of the five NCEP (ATP III) inclusion criteria: abdominal obesity, with a waist circumference of ≥102 cm in men or of ≥88 cm in women, serum TG of ≥150 mg/dl or drug treatment for elevated TG, serum HDL-c < 40 mg/dl in men and <50 mg/dl in women or drug treatment for low HDL-c, BP ≥ 130/85 mmHg or drug treatment for elevated BP, FG ≥ 100 mg/dl or drug treatment for elevated blood glucose.

Individuals were included in the patient group if they fulfilled the diagnostic criteria for hypertension (systolic BP > 130 mmHg, diastolic BP > 85 mmHg; at least two abnormal readings at two different visits over a period of 2 weeks) according to the existing guidelines (Chobanian et al., 2003). Patients were diagnosed with MS if they fit the criteria stipulated by the NCEP (ATP III) (Alberti et al., 2009). Patients were considered as having DIOS if they complied with increased serum ferritin (>350 μg/l in men and >250 μg/l in women) in the presence of normal TSAT (25–45%) and in the absence of any other cause of iron overload or inflammation (Mendler et al., 1999).

Exclusion criteria

Patients were excluded if they presented a history and/or clinical examination that suggested acute infection, or if they suffered from any cause of secondary hypertension. Moreover, exclusion criteria comprised pregnancy, or a history of drug abuse/medications (such as amphetamines, cocaine, sympathomimetics, systemic glucocorticoids, anabolic steroids, oral contraceptives, monoamine oxidase inhibitors, bromocriptine, erythropoietin, or non-steroidal anti-inflammatory drugs). Any case fitting the DIOS criteria but with a high C-reactive protein (CRP) or a low iron concentration was excluded.

All subjects underwent complete history taking and examination, including measurements of height, weight, and waist circumference. Sphygmomanometer-assisted measurement of the BP was also recorded.

Sampling

The circadian variation was considered by fixing the sampling time between 8:00–10:00 a.m., and all subjects were requested to fast for 12 hours prior to the analysis. In addition, patients were advised to have a complete physical and mental rest 15 minutes before sampling. Furthermore, the sampling was performed in a supine position. For aseptic venipuncture, 12 ml of blood was withdrawn and divided into 4 tubes; two serum separator tubes (4 ml) and two EDTA vacutainers (2 ml). The serum was left to clot for 15 minutes and then centrifuged at (1000 × g) for 10 minutes. Serum was harvested into three aliquots and kept at –80 °C until it was used in assays including FG, TG, HDL-c, cholesterol, uric acid, creatinine, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), CRP, iron profile (iron, ferritin, transferrin), and insulin.

The EDTA tubes were centrifuged, and the separated plasma was distributed into two aliquots in 2 ml sample vials with a cap (lot. 1041) as recommended by the manufacturer (RECIPE Chemicals, Munich, Germany) for storing at –80 °C until the adrenaline, noradrenaline, and dopamine were assayed by RIA.

Assay

Parameters assayed for the fulfillment of the NCEP (ATP III) criteria of MS

Measurements of FG, TG, HDL-c, and other markers assayed to exclude secondary diseases, such as cholesterol, uric acid, creatinine, ALT, and GGT, were performed using an ERBA XL 100 device (Mannheim, Germany), and the manufacturer's reagents. Moreover, as a marker of IR, fasting insulin was measured, and the homeostasis model assessment of insulin resistance index (HOMA-IR) equation was used as an indicator of IR. Insulin and ferritin were assayed using Cobas C601 (Roche Diagnostics, Indiana, USA).

Parameters assayed for the diagnosis of DIOS

The diagnosis of dysmetabolic iron overload was based on precise criteria (Alberti et al., 2009). This necessitated, apart from the ferritin assay previously mentioned, the measurement of iron (Iron Gen. 2) and transferrin (Tina-quant Transferrin ver. 2) on Cobas C601 (Roche Diagnostics, Indiana, USA) and a calculation of transferrin saturation. Additionally, to exclude hyperferritinemia secondary to an acute phase response, CRP levels were assayed using latex agglutination, and patients with levels exceeding 6 mg/dl were excluded (5 cases). Also, two cases presenting with an iron concentration of <15 μg/dl were excluded to avoid misinterpreting the transferrin saturation (TSAT) calculation.

Catecholamine level measurements

Catecholamines (adrenaline, noradrenaline, and dopamine) were assayed using the RIA (DIAsource ImmunoAssays S.A., Ottignies-Louvain-la-Neuve, Belgium).

Transferrin saturation (TSAT)

Normal TSAT (25–45%) (Mankad et al., 2012).

$$\text{TSAT} = \frac{\text{Serum iron}}{\text{serum transferrin} \times 100} \times 71.24$$

Homeostasis model assessment of insulin resistance (HOMA-IR)

Cut-off for IR > 3.8 (Qu et al., 2011).

$$\text{HOMA-IR} = \frac{(\text{fasting insulin}(\mu\text{U/ml}) \times \text{fasting blood sugar})(\text{mg/dl})}{405}$$

Statistical analysis

Data were analyzed using MedCalc© version 15 (MedCalc© Software Bvba, Ostend, Belgium). The normality of numerical data distribution was examined using the D'Agostino–Pearson test. The numerical variables were presented as mean ± SD, or median with interquartile ranges as appropriate. To analyze the between-group differences, the unpaired student's *t* test or the Mann–Whitney *U* test was used as appropriate. Categorical variables were presented as numbers and percentages, and differences were compared with Fisher's exact test.

Multivariable binary logistic regression analysis was used to identify independent predictors of DIOS. Variables significantly associated with DIOS by the univariable analysis were entered into a multivariable regression model, and the stepwise method was used to build up the final model. Probability (p) values < 0.05 were considered to indicate statistically significant differences.

Results

Among the 101 hypertensive patients included in the study (46 men and 55 women), 6 met the DIOS criteria. Fig. 1 displays the prevalence of MS among the hypertensive participants and the prevalence of DIOS.

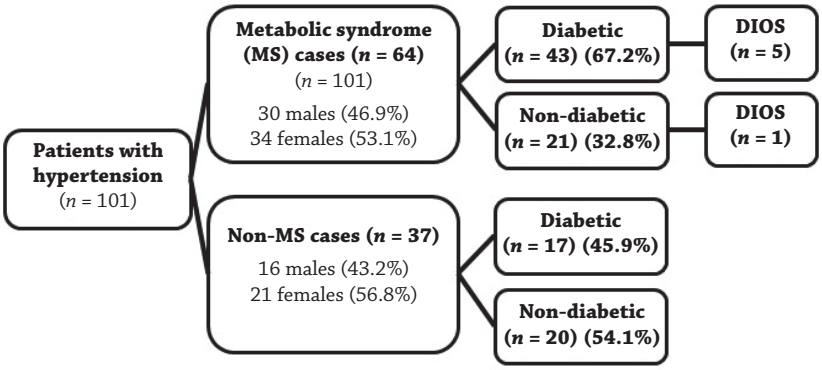

Fig. 1. The prevalence of metabolic syndrome (MS) and dysmetabolic iron overload syndrome (DIOS) among the participants of the study

The characteristics of patients with DIOS compared with those without are described in Table 1 (parametric variables), and Table 2 (non-parametric variables). In general, patients with DIOS were significantly older and had lower BMI than non-DIOS cases with (P = 0.026) and (P = 0.033), respectively, and those MS without DIOS in particular with (P = 0.003) and (P = 0.005), respectively. However, other parameters did not differ significantly between hypertensive patients with and without DIOS, including systolic BP, diastolic BP, height, weight, waist, FG, uric acid, cholesterol, and TG. Moreover, no statistically significant difference was observed between the catecholamine levels of the two groups.

Table 3 displays the features of the six patients with DIOS together with data on adrenaline, noradrenaline, and dopamine levels.

The comparison of hypertensive patients with normotensive controls revealed a statistically significant difference between both groups regarding FG (p < 0.001), GGT (p < 0.001), and uric acid (p < 0.001), while no difference between the groups was observed regarding ALT (p = 0.559), creatinine (p = 0.467), cholesterol (p = 0.229), TG (p = 0.175), and CRP (p = 0.791). Iron levels were significantly decreased in hypertensive patients (p = 0.043), while insulin and HOMA-IR were significantly increased; (p = 0.011) and (p < 0.01), respectively. Adrenaline and dopamine levels were significantly lower in hypertensive patients (p < 0.01) and (p = 0.020), respectively. No significant difference was observed between the groups regarding ferritin (p = 0.819), transferrin (p = 0.719), transferrin saturation (p = 0.054), or noradrenaline (p = 0.082).

Stepwise binary logistic regression analysis for predictors of DIOS in hypertensive patients revealed that age in years was the only statistically significant variable retained in the final model (p = 0.035) (Table 4). BMI, systolic BP, diastolic BP, and MS were excluded from the model.

Table 1. Demographic characteristics of patients with DIOS versus non-DIOS hypertensive patients (parametric variables)

Variable	No DIOS (n = 95)		DIOS (n = 6)		t	P-value
	Mean	SD	Mean	SD		
Age (years)	46	11	57	9	–2.268	0.026
BMI (kg/m^2)	33.7	6.2	28.2	2.5	2.168	0.033

BMI – body mass index; DIOS – Dysmetabolic Iron Overload Syndrome; SD – standard deviation; t – t statistic.

Table 2. Laboratory characteristics of patients with DIOS versus non-DIOS hypertensive patients (parametric variables)

Variable	No DIOS (n = 95)		DIOS (n = 6)		P-value
	Mean	IQR	Mean	IQR	
Iron (μg/dl)	61	44.0–80.0	77	63.0–97.0	0.189
Ferritin (μg/l)	75.6	38.3–150.5	394.4	295.8–550.5	<0.01
Transferrin saturation (%)	17.2	11.8–24.1	20.6	20.2–30.3	0.087
Insulin (μU/ml)	21	12–44	40	10–61	0.438
HOMA-IR	8	4.0–18.7	11.2	5.1–32.0	0.389
Adrenaline (pg/ml)	46.6	21.7–91.3	103	25.0–117.1	0.405
Noradrenaline (pg/ml)	251.8	207.7–425.7	264.7	183.7–382.5	0.687
Dopamine (pg/ml)	30	16 – 68	63	19–85	0.516

DIOS – Dysmetabolic Iron Overload Syndrome; HOMA-IR – Homeostasis Model Assessment Of Insulin Resistance; IQR – Interquartile Range.

Table 3. Clinical and laboratory features of the 6 cases with DIOS

Case no.	Age	Sex	BMI	Ferritin	Iron	TSAT	HOMA-IR	CRP	Adrenaline	Noradrenaline	Dopamine
5	58	F	26.6	291.7	63	20.5	32.2	N	103.7	183.7	66.6
22	46	F	28.1	550.5	50	21.3	9.108	N	120.6	187	85.22
40	60	F	28.5	295.8	87	20.1	107.2	N	117	176.2	159.2
43	50	F	32.6	441.4	97	30.3	13.28	N	108.7	382.5	60.17
71	55	M	27.7	740.6	120	42.9	3.497	N	25	342.3	18.8
87	71	M	25.2	350.4	67	20.5	5.102	N	15.07	735.6	8.146

BMI – Body Mass Index; CRP – C-Reactive Protein; F – female; HOMA-IR – Homeostasis Model Assessment of Insulin Resistance; M – male; N – negative; TSAT – Transferrin Saturation.

Table 4. Results of stepwise binary logistic regression analysis for predictors of DIOS in hypertensive patients

Variables retained in the model	P-value	Odds ratio (OR)	95% CI for OR
Age (years)	0.035	1.084	1.006 to 1.168

Discussion

The results of the current study revealed a prevalence of DIOS of 5.9%, and 9.3%, among hypertensive patients, and MS cases, respectively. Five out of 6 DIOS patients were diabetic. Such findings add support to the relationship between iron overload and IR suggested previously (Dongiovanni et al., 2011; Ruivard et al., 2009).

DIOS patients were older when compared to hypertensive patients without DIOS. A similar observation was previously reported in another study (Rametta et al., 2016). In the present cohort, age was the only significant predictor of DIOS, while other factors, such as BMI, BP, and MS, were not retained in the final regression model. Our results indicated that age might be a risk factor for DIOS. An explanation for why DIOS patients are older could be related to the fact that hepatic and visceral accumulation of iron requires years to build up (Adams et al., 2015).

Interestingly, BMI in our cohort of DIOS patients was significantly lower than in the hypertensive patients without DIOS. It was also lower than MS cases without DIOS. This appears to contradict the expectation that the metabolic features (obesity being one of them) would be worse in patients with DIOS than in their hypertensive non-DIOS counterparts.

Except for ferritin (which is a defining criterion of DIOS), all the other assayed parameters did not differ between DIOS and non-DIOS hypertensive patients.

In view of their role as an indicator of sympathetic activity (Navar, 2010), catecholamine levels did not differ between patients with DIOS and non-DIOS hypertensive patients. We refute a generalization of increased catecholamine levels among DIOS-affected patients.

A group of researchers have measured noradrenaline levels and revealed that noradrenaline was higher among hypertensive patients than among controls (Grassi et al., 2007). However, that result was contradicted by the same group in a later study (Grassi et al., 2008), in which, concomitant with the noradrenaline assay, micro-neurographic assessment of muscle sympathetic nerve activity was included as evidence of sympathetic drive. And although the muscle sympathetic nerve activity displayed an evident increase, the noradrenaline assay failed to reproduce these results.

According to the current data, there is no role for catecholamines to distinguish between hypertension with or without DIOS. To our knowledge, this report might be the first to address this point. Further studies are required in this aspect.

Thus, DIOS was disclosed in 9.3% of our cases. In DIOS, patients BMI was statistically lower, and age was statistically higher compared to non-DIOS patients. This puts a challenge not to miss this category of MS patients. Nevertheless, iron profiling is highly recommended for the early diagnosis of iron overload, which may result in detrimental consequences of liver cirrhosis and hepatocellular carcinomas.

Conclusions

We evaluated the association between plasma catecholamine status and hypertension in DIOS. DIOS patients were significantly older and had lower BMI compared with hypertensive non-DIOS patients. Adrenaline, noradrenaline, and dopamine levels did not differ significantly between DIOS and non-DIOS patients. Among MS cases, 9.3% had DIOS. Therefore, to prevent future complications, complete iron profiling should be routinely performed in MS cases for early diagnosis of DIOS.

Conflict of interests

The authors have no conflict of interests to declare.

Ethics approval and consent to participate

Data on patients were retrospectively collected in accordance with the guidelines of the Declaration of Helsinki.

Consent for publication

The study was approved by the Ethical Committee of the Faculty of Medicine, Cairo University. Informed consent was obtained from all patients, in accordance with the Declaration of Helsinki.

Authors' contributions

F.E. conceived and planned the study in consultation with H.N.B., and H.E.E. Then, F.E., H.N.B. and H.W.Z.H. designed the study and performed the analysis and statistics. H.W.Z.H. collected the samples, approvals and consent from patients, and the details and follow-up of cases. H.N.B. and H.W.Z.H. supervised the samples' preparation. H.W.H., L.F.A. and H.N.B., wrote the paper and prepared the tables and the figure. All authors contributed to data interpretation and manuscript writing, subsequently reviewing and editing the manuscript.

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