Atherosclerosis 328 (2021) 38–43

Contents lists available at ScienceDirect

Atherosclerosis

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

Association between vascular endothelial dysfunction and the inflammatory marker neopterin in patients with classic congenital adrenal hyperplasia

Hekma Saad Farghaly [a], Kotb Abbass Metwalley [a], Duaa Mohamed Raafat [a], Ghada Mohamed Saied [c], Magda Farghali Gabri [b], Magdy Algowhary [d,*]

[a] Department of Pediatrics, Faculty of Medicine, Assiut University, Asyut, Egypt
[b] Department of Pediatrics, Faculty of Medicine, Aswan University, Aswan, Egypt
[c] Department of Clinical Pathology, Faculty of Medicine, Assiut University, Asyut, Egypt
[d] Department of Cardiovascular Medicine, Faculty of Medicine, Assiut University, Asyut, Egypt

ARTICLE INFO

Keywords:
21-Hydroxylase deficiency
Neopterin
Carotid intimal media thickness
Flow-mediated dilation
Endothelial dysfunction
Inflammation

ABSTRACT

Background and aims: Patients with congenital adrenal hyperplasia (CAH) are at increased risk of cardiometabolic abnormalities. We aimed to evaluate vascular endothelial dysfunction and its association with serum neopterin (NP) levels in CAH patients.

Methods: The study included 40 patients, with a mean age of 14.8 ± 2.6 years; 28 (70%) subjects were females. They were compared with 40 healthy controls matched in anthropometric evaluation and measurement of fasting lipids, glucose, insulin, homeostasis model assessment for insulin resistance [HOMA-IR], and serum NP levels (nmol/L). Vascular ultrasound was used to measure brachial artery flow-mediated dilation (FMD%) and carotid intima-media thickness (CA-IMT). According to the degree of control on medical treatment, patients were classified into poor (n = 12) and good (n = 28) control groups.

Results: Compared to controls, CAH patients had lower brachial FMD% (4.60 ± 2.13 *versus* 9.31 ± 2.29, p = 0.001), similar CA-IMT (0.44 ± 0.08 *versus* 0.44 ± 0.06, p = nonsignificant) and higher NP (42.6 ± 11.6 *versus* 9.2 ± 3.8, p = 0.001). However, differences between poor and good control CAH patients were significant regarding FMD%, CA-IMT, and NP measurements. FMD% correlated significantly with NP (r = −0.54, p = 0.001), high-sensitivity CRP (r = −0.53, p = 0.001), HOMA-IR (r = −0.31, p = 0.01), CA-IMT (r = −0.22, p < 0.05), diastolic blood pressure (r = 0.32, p = 0.01) and systolic blood pressure (r = −0.022, p < 0.05). NP was the most significant independent predictor of FMD%, as determined by linear regression analysis (p = 0.001).

Conclusions: Our study showed that CAH patients had endothelial dysfunction, which is an early process of vascular affection. This was significantly associated with NP levels, suggesting a crucial role of inflammation in the pathogenesis of vascular damage. Further studies are needed to confirm our findings and to investigate the exact role of NP, as either protective or proatherothrombotic.

1. Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive condition resulting from mutations in enzymes required for adrenal steroid synthesis. Classical CAH occurs in 1:13,000 to 1:15,000 live births and defects in enzyme 21-hydroxylase (21OHD) are responsible for approximately 95% of cases [1]. It is estimated that 75% of patients have the salt-wasting (SW) phenotype and the rest have the simple-virilizing (SV) phenotype [2]. Patients with the classic form of CAH exhibit androgen excess with or without SW. In both forms, patients are exposed for long periods to high concentrations of corticotrophin-releasing hormone, adrenocorticotrophic hormone (ACTH), steroid precursors and adrenal androgens. They are at increased risk of cardiometabolic abnormalities [3], including obesity [4], insulin resistance, impaired glucose tolerance [5], gestational diabetes [6], hypertension, dyslipidaemia, hyperhomocysteinemia, and increased carotid artery intima-media thickness (CA-IMT) [7,8]. Of note, cardiovascular disease is the second most common cause of death

* Corresponding author.
E-mail address: magdyalgowhary@aun.edu.eg (M. Algowhary).

https://doi.org/10.1016/j.atherosclerosis.2021.05.017
Received 9 December 2020; Received in revised form 25 April 2021; Accepted 20 May 2021
Available online 27 May 2021

in CAH patients after adrenal crisis [9]. Life-long glucocorticoid therapy is mandatory to suppress the overproduction of ACTH and adrenal androgen and to restore normal growth. However, if therapy is not optimized, patients may develop either hyperandrogenism or hypercortisolism, aggravating cardiovascular risk [1,10,11].

Endothelial dysfunction is an early and reversible key event of vascular affection and has been used to predict future coronary artery disease prior to atherosclerotic changes [12]. Flow-mediated dilation (FMD) is a noninvasive ultrasound method currently recognized as a useful technique for the evaluation of endothelial function [13]. Additionally, neopterin (NP), a product of guanosine triphosphate produced by macrophages when stimulated by interferon-gamma (IFN-gamma) secreted by activated T-lymphocytes [14], is a novel biomarker for detection of the inflammatory response. NP may play a role in vascular dysfunction and atheromatous plaque formation [15]. Its vascular role on atherosclerotic processes, either beneficial or deleterious, is still under investigation. Moreover, its association with vascular dysfunction in CAH patients has not yet been validated.

The aim of the study was to assess vascular endothelial dysfunction in patients with CAH and to study its association with the biomarker NP levels.

2. Patients and methods

2.1. Study population

The study was a matched case-control study and included 40 CAH patients (28 females and 12 males), aged 9–16 years (mean 14.8 ± 2.6 years). All patients were affected by classic CAH (21OHD), 30 patients had SW (22 females, 8 males) and 10 patients had SV. All patients were recruited from the outpatient clinic of the Department of Paediatric Endocrinology of our University Hospital during their regular 3–6 month follow-up appointments between January 2018 and December 2018. The diagnosis of CAH was made based on clinical signs and biochemical assessment [elevated levels of ACTH, 17-hydroxyprogesterone (17-OHP), dehydroepiandrosterone, androstenedione, and testosterone, as well as low cortisol levels]. The SW form was diagnosed in patients with frank hyponatremia and hyperkalemia accompanied by low plasma aldosterone and elevated renin concentrations [16]. The mean duration of hydrocortisone therapy was 11.3 ± 2.2 years. It was given 3 times daily (50% of the daily dosage was given early in the morning and the remaining doses were given equally at noon and in the evening). Fludrocortisone therapy at a dose of 50–100 μg/m^2/day was also given [11]. The efficacy of the therapy was monitored periodically based on clinical and laboratory data according to clinical practice guidelines [1]. The exclusion criterion was subjects younger than 8 years, to ensure cooperation with ultrasound tests. None of the patients used chronic treatments other than steroids. Patients with known comorbidities such as liver failure, kidney failure, heart disease, other concomitant chronic diseases, and recurrent adrenal crisis in the past 5 years prior to enrolment were excluded. Forty healthy children matched for age, gender, pubertal status, and socioeconomic status were enrolled as controls. They were recruited from healthy children coming for a routine visit or vaccination. According to the degree of control of clinical and laboratory manifestations on medical treatment, patients with CAH were classified into 2 groups as poorly controlled patients (12 patients) who had signs of androgen excess, increased growth velocity, advanced bone age, and serum 17- hydroxyprogesterone levels >10 nmol/l and well-controlled patients (28 patients) who had serum 17-hydroxyprogesterone values <10 nmol/l [1]. The study protocol was approved by the ethical committee of our Children University Hospital in accordance with the Declaration of Helsinki, and written informed consent was obtained from the parents/guardians of all the participants.

Demographic and clinical data, including age, sex, type of congenital adrenal hyperplasia, mean hydrocortisone dosage (mg/m^2/day) in the last year, weight, weight standard deviation (SD) score, height, height SD score, body mass index, body mass index SD score (BMI-SDS), blood pressure, heart rate, and pubertal status were collected. Blood pressure was measured 3 times using an electronic sphygmomanometer (Omron HEM 712C, Kyoto, Japan) [17], and the mean measurement was recorded. Height was measured without shoes to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Croswell, Crymych, UK). Weight was measured using a digital scale to the nearest 0.1 kg, wearing light clothing and without shoes. The BMI-SDS was calculated according to our local reference standards [18]. Pubertal status was assessed according to Tanner staging [19]. Bone age was calculated from the X-ray of the left hand and wrist using the Greulich and Pyle method [20].

2.2. Laboratory investigations

Blood samples were drawn after an overnight fast of at least 12 h between 8:00–9:00 a.m. before the first dose of steroids for assessment of the serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose, and insulin. Lipids were measured by the enzymatic colorimetric method using a Hitachi 911 autoanalyser (Boehringer Mannheim, Indianapolis, IN, USA). Insulin levels were measured using the electrochemiluminescence immunoassay "ECLIA" (Roche Elecsys 1010/2010 and Modular Analytics E170, Roche Diagnostics GmbH, Mannheim, USA). Insulin resistance (IR) was calculated using the homeostasis model assessment (HOMA-IR) equation formula as follows: HOMA-IR = fasting insulin (μU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5. Patients were considered to have IR if HOMA-IR ≥2.6 [21]. 17-OHP and testosterone concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) [DRG 17-α-OH Progesterone ELISA (EIA-1292), Germany]. Total testosterone levels were measured by chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA, USA). Plasma high-sensitivity C-reactive protein (hs-CRP) was measured by enzyme immunoassay (catalogue no. E29-056; Immunospec Corp., Canoga Park, CA, USA). Determination of serum levels of neopterin was performed by enzyme-linked immunosorbent assay (ELISA) using a kit supplied by IBL International GmbH, Hamburg, Germany, according to the manufacturer's instructions.

2.3. FMD measurement

Ultrasound examination of the brachial artery was performed 5–10 cm above the antecubital fossa. The vessel diameter was measured at baseline and then an arm cuff was inflated to exceed systolic blood pressure. After 5 min, the cuff was deflated and the vessel diameter was measured (hyperemic stage). FMD% = (mean hyperemic vessel diameter – baseline vessel diameter)/baseline vessel diameter [22].

2.4. Measurement of carotid IMT

Carotid scanning was performed in the morning between 7:30 and 9:30 a.m. after overnight fasting. It was performed using a colour duplex flow imaging system (Acuson 128 XP; Acuson Corporation, Mountain View, CA, USA), which operates in several modes: real-time B, colour Doppler, and spectral Doppler modes. The patients were placed in a supine position with their necks slightly extended and their heads turned 45° away from the examination side. The probe was placed longitudinally in the anterolateral position first on the right side of the neck and then on the left side of the neck. Right CA-IMT was measured at 1 cm below the bifurcation of the carotid artery. The distance between the echogenicity of the lumen-intima interface and the adventitia-media interface was recorded as the thickness of the intima-media [23]. Three measurements at 3-mm intervals were taken. CA-IMT was calculated as the mean of three independent measurements from each side of the neck [24]. The images were analysed by independent trained readers who were blinded to clinical details. CA-IMT and FMD in all cases and

controls were measured by a well-trained cardiologist who was blinded to the clinical details. CA-IMT and FMD in all cases and controls were measured by a well-trained cardiologist who was blinded to the clinical details.

2.5. Statistical analysis

All statistical analyses were carried out using SPSS version 18.0 (Chicago, IL, USA). Quantitative variables are presented as the mean (±SD), and qualitative variables are presented as frequency and percentages (%). The Kolmogorov-Smirnov test was used to assess normality in the distribution of data. Comparisons of normal variables were made by using a *t*-test. Categorical variables were compared using the chi-square test with Fisher's exact method. Correlations were assessed by using Pearson test. Linear regression analysis using the stepwise method was used to determine factors that were significantly associated with FMD%. Two-sided $p < 0.05$ was considered statistically significant.

3. Results

Table 1 describes the anthropometric, biochemical, and hormonal characteristics of CAH patients and healthy controls. Both were comparable in age, gender distribution, and lipid profile (total cholesterol, triglycerides, LDL-C, and HDL-C). However, CAH patients had higher BMI-SDS, advanced bone age, and higher systolic and diastolic blood pressures. Although fasting insulin concentrations and HOMA index were within normal values, they were significantly higher in CAH patients than in controls. Of note, 17-OHP and testosterone levels were significantly higher in CAH patients than in controls, $p = 0.001$ for each. Moreover, NP, hs-CRP, and FMD% were significantly different, $p = 0.001$ for each, while CA-IMT was not significantly different (Table 2).

Table 3 shows the differences between CAH patients according to the response to medical treatment. Patients with poor control had significantly higher measurements of NP levels, hs-CRP, blood pressure (systolic and diastolic), HOMA-IR, testosterone levels, and CA-IMT, but lower FMD% compared to good control patients.

FMD% had moderate negative correlations with NP ($r = -0.54$, $p = 0.001$) and hs-CRP levels ($r = -0.53$, $p = 0.001$). Additionally, it was significantly correlated with age, blood pressure (systolic and diastolic), HOMA-IR, testosterone levels, and CA-IMT, but the strength of the relationship was lower (Table 4).

By multivariate regression analysis, the significant independent predictors of FMD% were NP levels (standardized coefficient 0.62, $p = 0.001$), testosterone levels (standardized coefficient 0.59, $p = 0.001$), hs-CRP levels (standardized coefficient 0.43, $p = 0.04$), systolic blood pressure (standardized coefficient 0.38, $p = 0.03$), and diastolic blood

Table 1
Anthropometric, biochemical, and hormonal characteristics of the studied groups.

	CAH (n = 40)	Controls (n = 40)	*p* value
Age (years)	14.80 ± 2.6	14.30 ± 2.7	NS
Male/Female	12/28	14/26	NS
SDS-BMI	1.02 ± 0.92	- 0.24 ± 1.5	0.001
SBP (mmHg)	119.0 ± 8.0	106.0 ± 7.0	0.001
DBP (mmHg)	75.0 ± 5.0	65.0 ± 5.0	0.001
Heart rate (beat/minute)	87 ± 8	83 ± 6	NS
Bone age; years	15.6 ± 2.4	13.1 ± 0.8	0.01
Total cholesterol (mg/dl)	155 ± 12.7	152 ± 9.5	NS
Triglycerides (mg/dl)	89.3 ± 20	80.1 ± 30	NS
LDL-C (mg/dl)	87.6 ± 13.4	82.9 ± 9.5	NS
HDL-C (mg/dl)	46 ± 5.2	43 ± 4.6	NS
Fasting blood glucose (mg/dl)	92.4 ± 15.8	81.6 ± 12.9	0.01
Fasting insulin (IU/mL)	14.2 ± 6.2	8.6 ± 2.8	0.001
HOMA-IR	3.3 ± 1.2	1.7 ± 0.8	0.001
17-OHP, nmol/l	189.2 ± 54.9	2.85 ± 0.95	0.001
Testosterone, ng/dl	518.3 ± 195.7	191.6 ± 62.5	0.001

Data are expressed as mean ± SD.
17-OHP, 17-hydroxyprogesterone; CAH, congenital adrenal hyperplasia; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; NS, nonsignificant; SBP, systolic blood pressure; SDS-BMI, standard deviation scores of body mass index.

Table 2
CA-IMT and FMD% values in the studied groups.

	CAH (n = 40)	Controls (n = 40)	*p* value
hs-CRP (mg/L)	3.8 ± 1.9	1.4 ± 0.5	0.001
Neopterin (nmol/L)	42.6 ± 11.6	9.2 ± 3.8	0.001
CA-IMT (mm)	0.44 ± 0.08	0.44 ± 0.06	NS
FMD (%)	4.60 ± 2.13	9.31 ± 2.29	0.001

Data are expressed as mean ± SD.
CAH, congenital adrenal hyperplasia; CA-IMT, carotid intima-media thickness; FMD%, percentage of flow-mediated dilation; hs-CRP, high sensitivity C-reactive protein; NS, nonsignificant.

Table 3
Demographics, laboratory data, CA-IMT, and FMD% of patients according to the degree of control on medical treatment.

	Patients with poor control (n = 12)	Patients with good control (n = 28)	*p* value
Age (years)	15.2 ± 0.8	13.1 ± 2.5	0.01
Bone age (years)	16.1 ± 1.2	13.3 ± 2.2	0.01
SBP (mmHg)	126.0 ± 7.0	112.0 ± 8.0	0.01
DBP (mmHg)	79.0 ± 6.0	68.0 ± 5.0	0.01
HOMA-IR	4.6 ± 1.9	2.8 ± 1.2	0.001
17-OHP (nmol/l)	284 ± 32.5	7.2 ± 1.3	0.001
Testosterone (ng/dl)	875.8 ± 212.6	183 ± 12.7	0.001
hs-CRP (mg/L)	6.7 ± 2.1	4.4 ± 1.6	0.001
Neopterin (nmol/L)	36.6 ± 6.2	12.2 ± 2.8	0.001
CA-IMT (mm)	0.46 ± 0.04	0.43 ± 0.02	0.05
FMD (%)	2.4 ± 1.1	6.7 ± 2.3	0.001

Data are expressed as mean ± SD.
17-OHP, 17-hydroxyprogesterone; CA-IMT, carotid intima-media thickness; DBP, diastolic blood pressure; FMD%, percentage of flow-mediated dilation; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; SBP, systolic blood pressure.

Table 4
Correlations between neopterin and FMD% with various confounding variables among CAH patients (n = 40).

Variables	Neopterin (r and *p* values)	FMD% (r and *p* values)
Age	0.298[a]	−0.321[a]
SDS-BMI	0.277[a]	−0.107
SBP (mmHg)	0.441[b]	−0.223[a]
DBP (mmHg)	0.332[b]	−0.316[b]
Total cholesterol (mg/dl)	0.171	−0.116
Triglycerides (mg/dl)	0.133	−0.118
HDL-C (mg/dl)	0.092	0.133
LDL-C (mg/dl)	0.081	−0.117
Insulin (lU/mL)	0.411[b]	−0.134
HOMA-IR	0.523[c]	−0.306[b]
Hs-CRP (mg/L)	0.426[b]	−0.532[c]
Testosterone (ng/dl)	0.368[a]	−0.231[a]
CA-IMT (mm)	0.422[b]	−0.223[a]
Neopterin (nmol/L)	–	−0.537[c]
FMD (%)	−0.537[c]	–

CA-IMT, carotid intima media thickness; DBP, diastolic blood pressure; FMD%, percentage of flow-mediated dilation; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitivity C reactive protein; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; SDS-BMI, standard deviation scores of body mass index.
[a] $p < 0.05$; [b] $p = 0.01$; [c] $p = 0.001$.

pressure (standardized coefficient 0.32, $p = 0.07$) (Table 5).

4. Discussion

The main finding of this study was the detection of vascular endothelial dysfunction in patients with CAH especially in patients with poor control. Moreover, the detected endothelial dysfunction was significantly associated with elevated NP levels, thereby providing a useful biomarker to detect endothelial dysfunction early, prior to the development of chronic vascular diseases. To the best of our knowledge this is the first study that demonstrates the association between NP and endothelial dysfunction in patients with CAH. It is noteworthy that cardiovascular disease is the second most common cause of death in CAH after adrenal crisis [9]. As such, in-depth investigations are required to elucidate the nature of the pathophysiology of endothelial dysfunction, which constitutes an early stage of cardiovascular disease in this group of patients. At this stage, serum NP level is considered a useful biomarker of endothelial dysfunction, and further work is needed to direct treatment to either augment or antagonize its action.

Atherosclerosis is a systemic disease affecting blood vessels resulting from a chronic inflammatory response to vascular injury. Endothelial inflammation produces proinflammatory cytokines and adhesive molecules such as interleukin 6, monocyte chemotactic protein 1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), resulting in monocyte adhesion and infiltration of the vessel wall. This releases proinflammatory cytokines such as tumour necrosis factor-a (TNF-a) and interferon c (IFN-c). Monocyte-derived macrophages consume oxidized low-density lipoprotein forming foam cells and fatty streaks resulting in plaque formation affecting the coronary arteries, aorta, and carotid arteries. These atherosclerotic plaques were found to contain NPs that were released from activated macrophages [14]. Endothelial dysfunction occurs early before atherosclerotic plaque formation, resulting in disturbance of the normal homeostasis of vascular integrity. This was evident in our population, who suffered from a reduction in dilatation response (FMD%) in the absence of pathologic thickening of carotid intima-media, suggesting early vascular changes. However, the long-standing effect of hormonal disturbance on the vascular bed may lead to intimal thickening and the development of plaque formation. Of note, similar findings were reported previously by Harrington et al. [25]. They reported no significant carotid intima-media thickening despite endothelial dysfunction in their CAH patients whose mean age was 14.8 ± 3.2 years. Wierzbicka-Chmiel et al. [26] recruited older CAH patients with a mean age of 23.7 ± 3.8 years. Their CAH population had significant intima-media thickening affecting the common carotid and the common femoral arteries, although the patients were still younger than the known traditional risk factor for the influence of age on the development of atherosclerosis. These findings emphasize the importance of early detection of vascular dysfunction in this group of patients.

NP can be considered a useful biomarker for atherosclerotic activity predicting future adverse cardiovascular events [27]. Moreover, high levels of NP were detected in atherosclerotic plaques, likely playing an important role by exerting either pathogenic or protective roles. The proatherothrombotic effect on endothelial cells was implicated through the induction of the tissue factor-mRNA transcription increasing state and cellular expression of the adhesion molecules, ICAM-1 and VCAM-1 [28]. On the other hand, a recent counteracting atheroprotective effect was identified through suppression of TNF-α–induced mRNA expression of *MCP-1*, *ICAM-1*, and *VCAM-1* attenuating adhesion to human aortic endothelial cells. Moreover, formation and migration of macrophages foam cells and proliferation of vascular smooth muscle cells were all suppressed [29,30]. For the aforementioned reasons, NP levels are not only a biomarker of inflammation and endothelial dysfunction but also a therapeutic target requiring further intervention trials. Recently, NP levels were influenced by antihypertensive treatment for patients with endothelial dysfunction and hypertension. A reduction in blood pressure levels was associated with a decrease in NP levels [31].

Testosterone was significantly increased in our study population due to hydroxylase enzyme deficiency in CAH patients resulting in accumulation of 17-OHP and androstenedione, which are precursors of testosterone. Consequently, testosterone levels increased in the circulation. Elevated testosterone was more marked in patients with poor medical control of CAH. Data from a recent meta-analysis of FMD studies demonstrated a reduction in FMD after chronic therapy with testosterone in hypogonadal men [32]. Not only exogenous testosterone but also high free endogenous testosterone could induce endothelial dysfunction in postmenopausal women [33]. In contrast, a low level of testosterone found in Japanese men with atherosclerotic risk factors was associated with reduced FMD [34]. We did not observe low testosterone levels in our patients due to hydroxylase enzyme deficiency in CAH patients. Moreover, the absence of association by linear regression analysis between traditional risk factors (older patients, male sex, smoking, diabetes mellitus, and dyslipidaemia) and FMD was most likely because of the small sample size and a relatively short duration of therapy. However, the testosterone level was significantly associated with endothelial dysfunction supporting the effect of the hormonal profile on FMD. Moreover, it correlated positively with NP levels, which might aggravate endothelial dysfunction.

Although the mean blood pressure in CAH patients was not abnormally high, even in poorly controlled patients, it was significantly correlated with NP levels and inversely correlated with FMD%. This finding highlighted the key role of inflammation in the pathogenesis of vascular dysfunction in patients with hypertension and *vice versa* because endothelial dysfunction might be a cause or a consequence of elevated blood pressure [35]. Taking into consideration that antihypertensive drugs used in the trials had different mechanisms of action, they all reduced blood pressure, improved endothelial dysfunction [36] and lowered NP levels [31].

FMD was impaired in CAH patients and associated with elevated levels of both NP and hs-CRP linking the inflammatory process with the pathogenesis of endothelial dysfunction detected in our patients [5]. Under the influence of inflammation and oxidative stress, hs-CRP was significantly high in patients with metabolic syndrome [37,38]. Elevated levels of hs-CRP were a predictor of endothelial dysfunction [37]. Additionally, it might play an important role in the process and instability of atherosclerosis. It was thought to be released from the smooth muscle cells of atherosclerotic plaques [39] and to be involved in vascular damage. Vascular dysfunction was evident through multiple mechanisms, including downregulation of endothelial nitric oxide synthase, decreased production of endothelium-derived relaxing factor, increased adhesion molecules, complement activation, upregulation of engulfment of low-density lipoprotein by macrophages, induction of smooth muscle cell migration, proliferation, and neointima formation [40]. Recently, the ARIC study proved the association between hs-CRP and the development of peripheral arterial disease in the general population after a median of 17.4 years of follow-up [41].

Table 5
Linear regression analysis of the relation of FMD%.

Variables	Standardized coefficients	*p* value
Age (years)	0.22	0.34
Disease duration (years)	0.16	0.65
SDS-BMI	0.13	0.35
HOMA-IR	0.12	0.72
DBP (mmHg)	0.32	0.07
SBP (mmHg)	0.38	0.03
hs-CRP (mg/L)	0.43	0.04
Testosterone (ng/dl)	0.59	0.001
Neopterin (nmol/L)	0.62	0.001

DBP, diastolic blood pressure; FMD%, percentage of flow-mediated dilation; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; SDS-BMI, standard deviation scores of body mass index.

4.1. Limitations

There were several limitations in the present study: it was a small-sized study; TNF, IFN, and adhesion molecules were not measured; long-term follow-up of the effect of NP on cardiovascular events was not performed.

4.2. Conclusion

CAH patients suffer from endothelial dysfunction which is an early event of vascular damage preceding the development of atherosclerotic plaques and further cardiovascular events. NP, as an inflammatory marker, was elevated and was involved in the pathogenesis of endothelial dysfunction. However, its definitive role as a protective or a proatherosclerotic factor needs further investigation.

CRediT authorship contribution statement

Hekma Saad Farghaly: Conceptualization, Methodology, Resources, Investigation, Writing – original draft. **Kotb Abbass Metwalley:** Conceptualization, Methodology, Resources, Writing – original draft, Investigation, Writing – review & editing, Supervision. **Duaa Mohamed Raafat:** Data curation, Investigation, Methodology, Resources, Validation. **Ghada Mohamed Saied:** Investigation, Resources. **Magda Farghali Gabri:** Conceptualization, Methodology, Investigation, Resources. **Magdy Algowhary:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Mohammed Kotb Abbass Metwallay at Armed Forces College of Medicine, Cairo, Egypt, for his invaluable assistance throughout all steps of manuscript preparation.

References

[1] P.W. Speiser, W. Arlt, R.J. Auchus, L.S. Baskin, G.S. Conway, D.P. Merke, H.F. L. Meyer-Bahlburg, W.L. Miller, M.H. Murad, S.E. Oberfield, P.C. White, Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline, J. Clin. Endocrinol. Metab. 103 (11) (2018) 4043–4088, https://doi.org/10.1210/jc.2018-01865 (Erratum in: J. Clin. Endocrinol. Metab. 2019; 104(1): 39-40).

[2] S. Baumgartner-Parzer, M. Witsch-Baumgartner, W. Hoeppner, EMQN best practice guidelines for molecular genetic testing and reporting of 21-hydroxylase deficiency, Eur. J. Hum. Genet. 28 (10) (2020) 1341–1367, https://doi.org/10.1038/s41431-020-0653-5.

[3] H. Falhammar, L. Frisén, A.L. Hirschberg, C. Norrby, C. Almqvist, A. Nordenskjöld, A. Nordenström, Increased cardiovascular and metabolic morbidity in patients with 21-hydroxylase deficiency: a Swedish population-based national cohort study, J. Clin. Endocrinol. Metab. 100 (9) (2015) 3520–3528, https://doi.org/10.1210/JC.2015-2093.

[4] T.M. Völkl, D. Simm, C. Beier, H.G. Dörr, Obesity among children and adolescents with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency, Pediatrics 117 (1) (2006) e98–105, https://doi.org/10.1542/peds.2005-1005.

[5] E. Charmandari, M. Weise, S.R. Bornstein, G. Eisenhofer, M.F. Keil, G.P. Chrousos, D.P. Merke, Children with classic congenital adrenal hyperplasia have elevated serum leptin concentrations and insulin resistance: potential clinical implications, J. Clin. Endocrinol. Metab. 87 (5) (2002) 2114–2120, https://doi.org/10.1210/jcem.87.5.8456.

[6] A.L. Hirschberg, S. Gidlöf, H. Falhammar, L. Frisén, C. Almqvist, A. Nordenskjöld, A. Nordenström, Reproductive and perinatal outcomes in women with congenital adrenal hyperplasia: a population-based cohort study, J. Clin. Endocrinol. Metab. 106 (2) (2021) e957–e965, https://doi.org/10.1210/clinem/dgaa801.

[7] K.A. Metwalley, H.S. Farghaly, A. Abdelhamid, Homocysteine level in children with classic congenital adrenal hyperplasia: relationship to carotid intimal wall thickness and left ventricular function, Horm. Res. Paediatr. 90 (4) (2018) 228–235, https://doi.org/10.1159/000492900.

[8] K.A. Metwalley, H.S. Farghaly, T. Sherief, Left ventricular dysfunction and subclinical atherosclerosis in children with classic congenital adrenal hyperplasia: a single-center study from upper Egypt, Eur. J. Pediatr. 175 (3) (2016) 405–412, https://doi.org/10.1007/s00431-015-2634-1.

[9] H. Falhammar, L. Frisén, C. Norrby, A.L. Hirschberg, C. Almqvist, A. Nordenskjöld, A. Nordenström, Increased mortality in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency, J. Clin. Endocrinol. Metab. 99 (12) (2014) E2715–E2721, https://doi.org/10.1210/jc.2014-2957.

[10] J.I. Labarta, E. Bello, M. Ruiz-Echarri, C. Rueda, P. Martul, E. Mayayo, A. Ferrández Longás, Childhood-onset congenital adrenal hyperplasia: long-term outcome and optimization of therapy, J. Pediatr. Endocrinol. Metab. 17 (Suppl 3) (2004) 411–422.

[11] A. Bachelot, Z. Chakhtoura, A. Rouxel, J. Dulon, P. Touraine, Hormonal treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency, Ann. Endocrinol. 68 (4) (2007) 274–280, https://doi.org/10.1016/j.ando.2007.06.019.

[12] J.P. Halcox, A.E. Donald, E. Ellins, D.R. Witte, M.J. Shipley, E.J. Brunner, M. G. Marmot, J.E. Deanfield, Endothelial function predicts progression of carotid intima-media thickness, Circulation 119 (7) (2009) 1005–1012, https://doi.org/10.1161/CIRCULATIONAHA.108.765701.

[13] L.H.B. Tacito, A.C. Pires, J.C. Yugar-Toledo, Impaired flow-mediated dilation response and carotid intima-media thickness in patients with type 1 diabetes mellitus with a mean disease duration of 4.1 years, Arch. Endocrinol. Metab. 61 (6) (2017) 542–549, https://doi.org/10.1590/2359-3997000000281.

[14] G. Hoffmann, B. Wirleitner, D. Fuchs, Potential role of immune system activation-associated production of neopterin derivatives in humans, Inflamm. Res. 52 (8) (2003) 313–321, https://doi.org/10.1007/s00011-003-1181-9.

[15] D. Fuchs, P. Avanzas, R. Arroyo-Espliguero, M. Jenny, L. Consuegra-Sanchez, J. C. Kaski, The role of neopterin in atherogenesis and cardiovascular risk assessment, Curr. Med. Chem. 16 (35) (2009) 4644–4653, https://doi.org/10.2174/092986709789878247.

[16] P.C. Hindmarsh, Management of the child with congenital adrenal hyperplasia, Best Pract. Res. Clin. Endocrinol. Metabol. 23 (2) (2009) 193–208, https://doi.org/10.1016/j.beem.2008.10.010.

[17] American Academy of Pediatrics, National high blood pressure education program working group on high blood pressure in children and adolescents (The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents), Pediatrics 114 (2 Suppl 4th Report) (2004) 555–576.

[18] [Internet] Diabetes Endocrine Metabolism Pediatric Unit, Cairo University Children's Hospital, Egyptian growth curves, Available from: http://dempuegypt.blogspot.com, 2002 [Accessed 15 Aug 2017].

[19] J.M. Tanner, Growth at Adolescence, Blackwell Scientific Publications, Oxford, 1962.

[20] G.W. Greulich, S.I. Pyle, Radiographic Atlas of Skeletal Development of the Hand and Wrist, second ed., Stanford University Press, Stanford, 1959.

[21] R. Burrows, P. Correa-Burrows, M. Reyes, E. Blanco, C. Albala, S. Gahagan, Healthy Chilean adolescents with HOMA-IR $\geq$ 2.6 have increased cardiometabolic risk: association with genetic, biological, and environmental factors, J. Diabetes Res. (2015) 783296, https://doi.org/10.1155/2015/783296.

[22] H. Alley, C.D. Owens, W.J. Gasper, S.M. Grenon, Ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery in clinical research, JoVE 92 (2014) e52070, https://doi.org/10.3791/52070.

[23] A. Srámek, J.G. Bosch, J.H. Reiber, J.A. Van Oostayen, F.R. Rosendaal, Ultrasound assessment of atherosclerotic vessel wall changes: reproducibility of intima-media thickness measurements in carotid and femoral arteries, Invest. Radiol. 35 (12) (2000) 699–706, https://doi.org/10.1097/00004424-200012000-00001.

[24] M.J. Järvisalo, A. Putto-Laurila, L. Jartti, T. Lehtimäki, T. Solakivi, T. Rönnemaa, O.T. Raitakari, Carotid artery intima-media thickness in children with type 1 diabetes, Diabetes 51 (2) (2002) 493–498, https://doi.org/10.2337/diabetes.51.2.493.

[25] J. Harrington, A.S. Peña, R. Gent, C. Hirte, J. Couper, Adolescents with congenital adrenal hyperplasia because of 21-hydroxylase deficiency have vascular dysfunction, Clin. Endocrinol. 76 (6) (2012) 837–842, https://doi.org/10.1111/j.1365-2265.2011.04309.x.

[26] J. Wierzbicka-Chmiel, A. Chmiel, S. Rychlik, M. Ogrodowczyk-Bobik, B. Marek, D. Kajdaniuk, Vascular and cardiac function in young adults with classical congenital adrenal hyperplasia, Endokrynol. Pol. 68 (5) (2017) 505–511, https://doi.org/10.5603/EP.a2017.0046.

[27] M. Pacileo, P. Cirillo, S. De Rosa, G. Ucci, G. Petrillo, S. Musto D'Amore, L. Sasso, P. Maietta, R. Spagnuolo, M. Chiariello, The role of neopterin in cardiovascular disease, Monaldi Arch. Chest Dis. 68 (2) (2007) 68–73, https://doi.org/10.4081/monaldi.2007.454.

[28] P. Cirillo, M. Pacileo, S. De Rosa, P. Calabrò, A. Gargiulo, V. Angri, F. Granato-Corigliano, I. Fiorentino, N. Prevete, R. De Palma, C. Mauro, A. Leonardi, M. Chiariello, Neopterin induces pro-atherothrombotic phenotype in human coronary endothelial cells, J. Thromb. Haemostasis 4 (10) (2006) 2248–2255, https://doi.org/10.1111/j.1538-7836.2006.02125.x.

[29] R. Shirai, K. Sato, T. Yamashita, M. Yamaguchi, T. Okano, K. Watanabe-Kominato, R. Watanabe, T.A. Matsuyama, H. Ishibashi-Ueda, S. Koba, Y. Kobayashi, T. Hirano, T. Watanabe, Neopterin counters vascular inflammation and atherosclerosis, J. Am. Heart Assoc. 7 (3) (2018) e007359, https://doi.org/10.1161/JAHA.117.007359.

[30] T. Watanabe, Neopterin derivatives - a novel therapeutic target rather than biomarker for atherosclerosis and related diseases, Vasa 14 (2020) 1–9, https://doi.org/10.1024/0301-1526/a000903.

[31] Y.Y. Zhang, X.Z. Tong, W.H. Xia, W.L. Xie, B.B. Yu, B. Zhang, L. Chen, J. Tao, Increased plasma neopterin levels are associated with reduced endothelial function and arterial elasticity in hypertension, J. Hum. Hypertens. 30 (7) (2016) 436–441, https://doi.org/10.1038/jhh.2015.72.

[32] A. Sansone, G. Rastrelli, A. Cignarelli, M. de Rocco Ponce, R.A. Condorelli, E. Giannetta, E. Maseroli, S. Pinto, C. Salzano, D. Santi, Effect of treatment with testosterone on endothelial function in hypogonadal men: a systematic review and meta-analysis, Int. J. Impot. Res. 32 (4) (2020) 379–386, https://doi.org/10.1038/s41443-019-0163-6.

[33] L. Mathews, V. Subramanya, D. Zhao, P. Ouyang, D. Vaidya, E. Guallar, J. Yeboah, D. Herrington, A.G. Hays, M.J. Budoff, E.D. Michos, Endogenous sex hormones and endothelial function in postmenopausal women and men: the multi-ethnic study of atherosclerosis, J. Womens Health (Larchmt) 28 (7) (2019) 900–909, https://doi.org/10.1089/jwh.2018.7441.

[34] M. Akishita, M. Hashimoto, Y. Ohike, S. Ogawa, K. Iijima, M. Eto, Y. Ouchi, Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors, Atherosclerosis 210 (1) (2010) 232–236, https://doi.org/10.1016/j.atherosclerosis.2009.10.037.

[35] L.P. Santos, D. Umpierre, Exercise, cardiovascular health, and risk factors for atherosclerosis: a narrative review on these complex relationships and caveats of literature, Front. Physiol. 11 (2020) 840, https://doi.org/10.3389/fphys.2020.00840.

[36] Y. Shahin, J.A. Khan, N. Samuel, I. Chetter, Angiotensin converting enzyme inhibitors effect on endothelial dysfunction: a meta-analysis of randomised controlled trials, Atherosclerosis 216 (1) (2011) 7–16, https://doi.org/10.1016/j.atherosclerosis.2011.02.044.

[37] N.T. Sipahioglu, B. Ilerigelen, Z.B. Gungor, G. Ayaz, H. Ekmekci, C.B. Gurel, G. Can, H. Sonmez, T. Ulutin, F. Sipahioglu, Relation of biochemical parameters with flow-mediated dilatation in patients with metabolic syndrome, Chin. Med. J. 130 (13) (2017) 1564–1569, https://doi.org/10.4103/0366-6999.208231.

[38] J. Li, A.J. Flammer, R.J. Lennon, R.E. Nelson, R. Gulati, P.A. Friedman, R. J. Thomas, N.P. Sandhu, Q. Hua, L.O. Lerman, A. Lerman, Comparison of the effect of the metabolic syndrome and multiple traditional cardiovascular risk factors on vascular function, Mayo Clin. Proc. 87 (10) (2012) 968–975, https://doi.org/10.1016/j.mayocp.2012.07.004.

[39] P. Calabró, J.T. Willerson, E.T. Yeh, Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells, Circulation 108 (16) (2003) 1930–1932, https://doi.org/10.1161/01.CIR.0000096055.62724.C5.

[40] F.J. Khawaja, I.J. Kullo, Novel markers of peripheral arterial disease, Vasc. Med. 14 (4) (2009) 381–392, https://doi.org/10.1177/1358863X09106869.

[41] N. Ding, C. Yang, S.H. Ballew, C.A. Kalbaugh, J.W. McEvoy, M. Salameh, D. Aguilar, R.C. Hoogeveen, V. Nambi, E. Selvin, A.R. Folsom, G. Heiss, J. Coresh, C.M. Ballantyne, K. Matsushita, Fibrosis and inflammatory markers and long-term risk of peripheral artery disease: the ARIC study, Arterioscler. Thromb. Vasc. Biol. 40 (9) (2020) 2322–2331, https://doi.org/10.1161/ATVBAHA.120.314824.