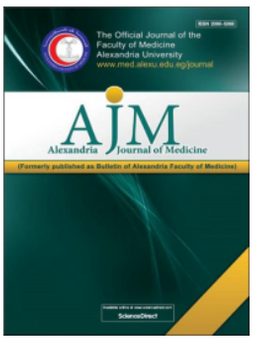

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Role of expression of atrial natriuretic peptide gene in essential hypertension among Egyptian patients

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ABSTRACT

Introduction: Essential Hypertension has been a great burden on public health services for a long time, with many life-threatening complications. Therefore, we decided to study Atrial Natriuretic peptide (ANP) gene expression as one of the most important blood pressure controlling genes, in order to use ANP gene as a potential diagnostic or therapeutic marker in the near future.

Methods: One hundred essential hypertensive patients and 100 normotensive controls were included. Study Subjects were subjected to ANP gene expression analysis, together with blood pressure measurement, Lab investigations, and BMI analysis.

Results: There was a statistical difference between ANP gene expression and blood pressure, with lower ANP gene expression level (median of 0.3) being present among hypertensive patients and higher ANP gene expression level (median of 1.6) among normotensive controls ($p < 0.001$).

Discussion: We proved that ANP gene expression to be low in essential hypertension patients compared with normotensive individuals.

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KEYWORDS
Hypertension; gene expression; ANP

1. Introduction

Arterial Essential Hypertension (EH) is a big global medical problem of high impact on healthcare resources [1,2]. It was estimated in 2010 that around 1.13 billion people suffer from hypertension worldwide [3]. EH represents 90% of the causes of increased blood pressure, with the remaining 10% due to secondary causes [4,5]. EH represents a major risk factor for serious cardiovascular and renal illnesses like heart failure, stroke, coronary heart disease, and atrial fibrillation [2,6]. Those suffering from EH have 2 to 3 folds higher risk for these cardiovascular illnesses compared to non-hypertensive subjects [7]. Nowadays, despite the recent treatment modalities available for hypertension, it is estimated that less than one in five patients with hypertension is under control [2].

EH has undetermined causes until now [5]. It has been well known that EH is multifactorial in origin, with several genes interacting with various environmental and lifestyle factors that result in increased blood pressure in susceptible patients [8,9]. Several factors have been proved to be involved in the development of hypertension, such as increased cardiac output and plasma volume [10], potentiated sympathetic activity [11], hyperinsulinemia, and insulin resistance [12], and nutritional factors such as high sodium consumption [13].

As many causes are implicated in EH pathogenesis, it can be said that the renin-angiotensin-aldosterone system (RAAS) is the most important cause [14,15]. The RAAS certainly plays an essential role in water and electrolyte homeostasis in the body. RAAS is composed of renin, angiotensinogen, angiotensinogen converting enzyme, angiotensin II type 1 receptor, aldosterone synthetase, and atrial natriuretic peptide (ANP) [16]. Renin cleaves angiotensinogen to angiotensin I (Ang I). Ang I is then converted to angiotensin II (Ang II) by angiotensin-converting enzyme (ACE) [17]. Ang II then exerts its action via Ang II type 1 and type 2 receptors (AT1 and AT2, respectively). AT1 is the receptor involved in the classic pathway of RAAS and is, subsequently, responsible for the development of hypertension. Ang II actions include vascular constriction, salt and water retention, hypertrophy, aldosterone release and stimulation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase leading to mitochondrial dysfunction and oxidative stress [18,19]. RAAS inhibitors, including ACE inhibitors, renin inhibitors, AngII type 1 receptor blockers (ARbs), have been introduced in hypertension

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treatment. But, although they are effective in controlling BP, their ability to prevent end-organ damage is limited and they have multiple side effects after prolonged use [2,20].

ANP is one of the essential components of the RAAS that controls blood pressure in EH [21]. ANP is an endogenous cardiac hormone that regulates natriuresis, homeostasis, and vasodilatation that, in turn, lower blood pressure and blood volume [22–25]. The ANP is initially secreted as an inactive pro-atrial natriuretic peptide (pro-ANP) that is converted into the active form of ANP by a transmembrane serine protease called Corin [26,27]. Activated ANP is a 28 amino-acid endogenous vasoactive peptide hormone, secreted predominantly by atrial cardiomyocytes [23].

ANP acts through binding to guanylyl cyclase natriuretic peptide receptor-1 (NPR-1) [28,29]. NPR-1 stimulation leads to rapid conversion of intracellular GTP into cGMP increasing its intracellular levels leading to activation of cGMP-dependent phosphodiesterases, cGMP-dependent protein kinases (PKG-I and PKG-II), and cGMP-dependent ion channels promoting sodium excretion and inducing vasodilatation [28–30].

ANP secretion is stimulated primarily by distension of atrial tissues in healthy persons [31] and to a lesser extent in ventricular myocytes, if exposed to severe tension [32]. The ventricular cells producing ANP rise significantly if the cardiac ventricles are pressure loaded and hypertrophied, as in the case of chronic hypertension. Therefore, ANP is considered a reliable marker of myocardial hypertrophy [33].

The molecular gene encoding ANP is one of the most developmentally and transcriptionally regulated genes. On the molecular biology level of cardiac cells, ANP encoding gene is one of the essential cardiac genes that led to the development of recent and novel research on gene regulation in the cardiovascular system [25]. In previous research, ANP gene polymorphisms were found to be affecting ANP gene expression levels and thus, linked to cardiovascular disturbances and illnesses like coronary heart disease and left ventricular failure [34].

As the burden of hypertension and its complications are greatly increasing, as well as the multiple adverse effects of the available medications, despite their efficacy, we decided to study the role of ANP gene expression in patients with EH as a new promising diagnostic and therapeutic marker for EH in the future.

2. Patients and methods

Our case-control study was approved by the Medical Ethics Committee of Alexandria Faculty of Medicine. We recruited 100 patients newly diagnosed with essential hypertension from the Internal Medicine department at Alexandria University hospitals, Egypt. Exclusion criteria included secondary hypertension, previously diagnosed hypertensive patients on treatment, diabetes mellitus, and renal failure. One hundred normotensive subjects of matched age and sex to the patients' group were recruited from the outpatients' clinic as the control group. All study subjects signed an informed consent showing the nature and type of the study.

Full history taking and thorough physical examination were done on all study subjects. The blood pressure was measured 3 times in the resting state in the supine position, with the average of the last readings being recorded. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg [35]. Routine laboratory tests were performed to all study subjects, as fasting plasma glucose (FPG), lipid profile, blood urea, serum creatinine, sodium, and potassium were assessed under overnight fasting conditions using Dimension RxL autoanalyzer. Height and weight were measured for all subjects to calculate body mass index (BMI). These tests and measurements were done because diabetes mellitus, hyperlipedemia, renal impairment, and obesity are well-known risk factors or associations of hypertension.

2.1. Genetic study

2.1.1. RNA extraction

RNA was isolated from the peripheral blood mononuclear cells according to the manufacturer's protocol using QIAamp RNA Blood Mini Kits (Qiagen, USA). The quality and quantity of the extracted RNA were detected using Nanodrop 2000 spectrophotometer (Nanodrop Technologies, USA). The extracted RNA was stored at −80 C until further processing.

2.1.2. Reverse transcription

Complementary DNA (cDNA) was then synthesized from the previously extracted RNA using SensiFAST cDNA Synthesis Kit (Bioline, London, UK) following the manufacturer's protocol for RNA reverse transcription. The PCR amplification was done on the SimpliAmp Thermal Cycler (Applied Biosystems, USA). The thermal profile was as follows: annealing at 25 C for 10 min, reverse transcription at 42 C for 15 min followed by inactivation at 85 C for 5 min.

2.1.3. ANP gene expression analysis

Quantitative detection of ANP gene expression level was carried out on Stratagene Mx3000P Real-time PCR system (Agilent, Germany) using presynthesized cDNA, Maxima SYBR Green PCR Master Mix (Thermo Scientific, USA) and sequence-specific primers. The reaction mix was prepared by adding 12.5 uL Maxima SYBR Green, 1 uL

Table 1. Clinicopathological features of study subjects.

	Patients (n = 100)	Controls (n = 100)	p
Age (years)			
Mean ± SD.	53.4 ± 8.2	52.3 ± 8.2	0.235
Median (Min. – Max.)	52 (40–68)	54 (40–67)	
Sex			
Male	75 (75%)	85 (85%)	0.077
Smoking	58 (58%)	47 (47%)	0.119
Family history of HTN	28 (28%)	26 (26%)	0.750
BMI (Kg/m^2)			
Mean ± SD.	23.4 ± 2.7	22.9 ± 2.9	0.154
Median (Min. – Max.)	23 (20–32)	22 (18–29)	
FPG (mg/dL)			
Mean ± SD.	90.1 ± 7.7	88.2 ± 7	0.071
Median (Min. – Max.)	89 (79–105)	86 (79–102)	
Blood urea (mg/dL)			
Mean ± SD.	23.4 ± 4.4	23.4 ± 5.2	0.555
Median (Min. – Max.)	23 (18–36)	21 (18–36)	
Serum creatinine (mg/dL)			
Mean ± SD.	0.7 ± 0.3	0.7 ± 0.3	0.949
Median (Min. – Max.)	0.6 (0.2–1.4)	0.6 (0.2–1.4)	
Total cholesterol (mg/dL)			
Mean ± SD.	165.6 ± 30.9	165.6 ± 30.9	1.000
Median (Min. – Max.)	165 (112–205)	165 (112–205)	
HDL-C (mg/dL)			
Mean ± SD.	44.1 ± 11.1	45.2 ± 10.7	0.381
Median (Min. – Max.)	45 (30–67)	45.5 (30–81)	
LDL-C (mg/dL)			
Mean ± SD.	99.4 ± 19	107.4 ± 17.4	<0.001*
Median (Min. – Max.)	100 (58–147)	107 (65–147)	
Triglycerides (mg/dL)			
Mean ± SD.	123.2 ± 21.8	122.1 ± 24	0.939
Median (Min. – Max.)	123 (65–155)	124 (65–155)	
Sodium (mmol/L)			
Mean ± SD.	140.8 ± 5.1	140.4 ± 5.4	0.415
Median (Min. – Max.)	139 (135–149)	138 (135–149)	
Potassium (mmol/L)			
Mean ± SD.	4 ± 0.4	3.9 ± 0.4	0.224
Median (Min. – Max.)	4.1 (3.5–4.7)	4 (3.5–4.7)	
SBP (mmHg)			
Mean ± SD.	161.7 ± 14.7	118.8 ± 5.6	<0.001*
Median (Min. – Max.)	160 (141–190)	120 (110–125)	
DBP (mmHg)			
Mean ± SD.	98 ± 6.5	79.5 ± 5.2	<0.001*
Median (Min. – Max.)	100 (87–110)	80 (70–89)	
ANP gene expression			
Mean ± SD.	0.5 ± 0.6	6.1 ± 7.5	<0.001*
Median (Min. – Max.)	0.3 (0.1–2.4)	1.6 (0.1–26)	

χ^2: Chi square test U: Mann Whitney test SD: Standard deviation.
p: p value for comparing between Patients and Controls.
*: Statistically significant at p ≤ 0.05.

forward primer, 1 uL reverse primer, 5 uL template DNA and nuclease-free water of 5.5 uL to make a total volume of 25 uL. The forward primer for ANP gene was 5′-AGATAACAGCCAGGGAGGACAA-3′ and the reverse primer for ANP gene was 5′-AGGCGAGGAAGTCACCATCAA-3′. The thermal cycling conditions were as follows: initial denaturation step at 95°C for 10 min followed by 40 cycles that included 15 seconds at 95 C° for denaturation, 30 seconds at 60 C° for annealing and 30 seconds at 72 C° for an extension. 18S rRNA gene was used as the endogenous control with its expression being stable in all samples and independent of the analyzed variables. The forward primer for 18S rRNA gene was 5′-GTGGTGTTGAGGAAAGCAGACA-3′ and the reverse primer for 18S rRNA gene was 5′-TGATCACAGGTTCCACCTCATC-3′. Relative levels of ANP gene expression were calculated using the $2^{-\Delta\Delta}$CT method [36].

2.1.4. Statistical analysis

IBM SPSS software version 20 was used for data analysis. Values of $p<0.05$ were considered statistically significant. Quantitative data were expressed as mean ±standard deviation (SD). Student's *t*-test was use for comparison between 2 groups of normally distributed data. Comparisons between groups for categorical variables were assessed using the chi-square test. Mann–Whitney test was used for comparing every two groups. Correlation between various parameters was calculated using Pearson's test. ROC curve analysis was used to determine the sensitivity and specificity of ANP gene expression in differentiating hypertensives from normotensives.

3. Results

3.1. Demographic characteristics of hypertensive patients and normotensive controls

The baseline criteria of both groups of the study population can be checked in Table 1. The distribution of hypertensive patients and normotensive controls by age, gender, smoking status and BMI was similar and insignificant between the controls and the hypertensive patients. On the other hand, systolic and diastolic blood pressures were significantly increased among EH patients compared with controls (SBP: 161.7 ± 14.7 for patients vs 118.8 ± 5.6 for controls and DBP: 98 ± 6.5 for patients vs 79.5 ± 5.2 for controls) ($p < 0.001$). Regarding laboratory data, no statistical significance was detected between both study groups regarding blood glucose, blood urea, serum creatinine, total cholesterol, HDL-cholesterol, triglycerides, sodium, and potassium levels. Only LDL-cholesterol showed a significant difference between hypertensive patients and normotensive controls (99.4 ± 19 for patients vs 107.4 ± 17.4 for controls) ($p < 0.001$, Table 1).

3.2. ANP gene expression profile and its association with the clinical and laboratory findings

There was a statistical difference between ANP gene expression and blood pressure level, showing lower ANP gene expression level among hypertensive patients versus higher ANP gene expression level among normotensive controls ($p < 0.001$, Table 1, Figure 1).

Figure 2 represents the scatter plot between SBP and ANP, and DBP and ANP gene expression levels among hypertensive patients. It showed that there is a negative correlation between ANP gene expression level and both SBP ($r = -0.506$, $p < 0.001$) and DBP ($r = -0.561$, $p < 0.001$).

Hypertensive patients were divided into two groups by the median value of the ANP gene expression into a high expression-ANP group and low expression-ANP group

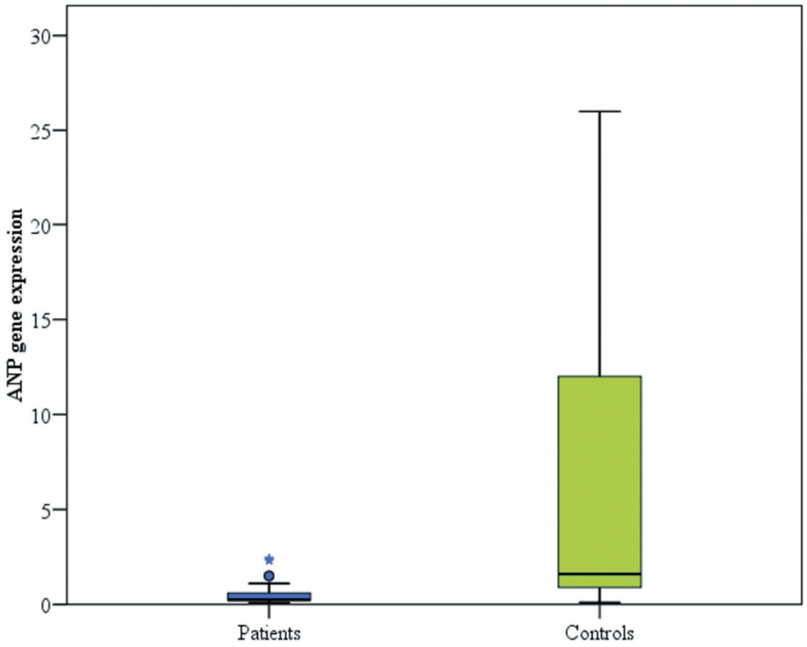

Figure 1. ANP gene expression between patients and controls.

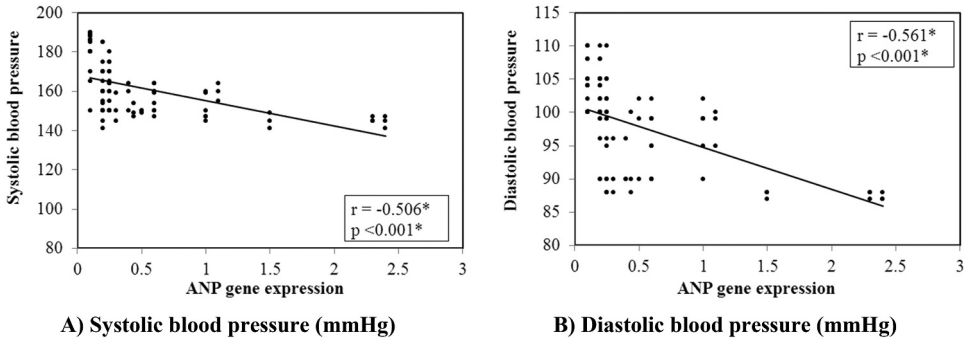

Figure 2. Correlation between ANP gene expression and systolic (a) and diastolic (b) blood pressure.

(Table 2). No statistical significance was observed between the different expression levels of ANP gene and other clinical variables, including age, gender, smoking status and BMI (Table 2).

There was an insignificant relationship between laboratory findings including blood glucose, blood urea, serum creatinine, LDL-cholesterol, HDL-cholesterol, triglycerides, sodium, potassium on one side and the different ANP gene expression levels on the other side, in both groups: patients and controls.

However, there was a significant correlation between SBP and DBP with the different expression levels of ANP gene expression ($p < 0.001$ and $p < 0.001$ respectively) (Table 2).

3.3. Analytical performance of ANP gene expression level

ROC curve analysis was used to determine the diagnostic performance of ANP gene expression in differentiating patients with hypertension from non-hypertensive controls. ANP expression at a cutoff value of ≤0.6 had 82% sensitivity and 88% specificity to detect increased blood pressure (Figure 3, Table 3).

4. Discussion

Arterial Essential Hypertension (EH) represents a challenging public health issue worldwide [1,2]. It predisposes to major public health problems like cardiovascular illnesses, neurological sequelae, and renal impairment [2,6]. Therefore, we decided to carry out this study in order to find out whether gene expressions of related proteins like ANP would be of value in diagnosing and managing hypertension in an earlier stage before complications occur, or if it could value in choosing treatment modalities.

In this study, hypertensive patients showed lower expression of ANP gene than normotensive controls. As we know, The RAAS system helps regulate blood pressure and homeostasis within the human body through various mechanisms involving hormones

Table 2. Relationship between ANP gene expression levels and different parameters in EH patients.

	ANP gene expression		
	Low (n = 61)	High (n = 39)	p
BMI (Kg/m^2)			
Mean ± SD.	23.3 ± 2.7	23.7 ± 2.5	0.295
Median (Min. – Max.)	23 (20–32)	24 (20–30)	
FPG (mg/dL)			
Mean ± SD.	90.9 ± 8	88.7 ± 7.1	0.190
Median (Min. – Max.)	90 (79–105)	87 (79–102)	
Blood urea (mg/dL)			
Mean ± SD.	23.6 ± 4.2	23 ± 4.7	0.227
Median (Min. – Max.)	24 (18–36)	21 (18–36)	
Serum creatinine (mg/dL)			
Mean ± SD.	0.7 ± 0.3	0.7 ± 0.4	0.494
Median (Min. – Max.)	0.6 (0.2–1.4)	0.5 (0.2–1.4)	
Total cholesterol (mg/dL)			
Mean ± SD.	164.1 ± 31	167.9 ± 31.1	0.574
Median (Min. – Max.)	165 (112–205)	178 (112–205)	
HDL-C (mg/dL)			
Mean ± SD.	45 ± 11.1	42.7 ± 11.2	0.172
Median (Min. – Max.)	45 (30–67)	45 (30–67)	
LDL-C (mg/dL)			
Mean ± SD.	100.3 ± 19.8	98 ± 18	0.608
Median (Min. – Max.)	100 (58–147)	99 (58–147)	
Triglycerides (mg/dL)			
Mean ± SD.	123.2 ± 21.8	123.2 ± 22.1	0.963
Median (Min. – Max.)	123 (65–155)	123 (65–153)	
Sodium (mmol/L)			
Mean ± SD.	140.6 ± 5	141 ± 5.4	0.725
Median (Min. – Max.)	139 (135–149)	139 (135–149)	
Potassium (mmol/L)			
Mean ± SD.	4 ± 0.4	3.9 ± 0.4	0.159
Median (Min. – Max.)	4.1 (3.5–4.7)	3.9 (3.5–4.7)	
SBP (mmHg)			
Mean ± SD.	162.3 ± 15	152 ± 6.9	<0.001*
Median (Min. – Max.)	165 (141–190)	150 (141–164)	
DBP (mmHg)			
Mean ± SD.	100.9 ± 5.5	93.4 ± 5.3	<0.001*
Median (Min. – Max.)	100 (88–110)	90(87–102)	

χ^2: Chi square test U: Mann Whitney test SD: Standard deviation.
*: Statistically significant at $p \leq 0.05$.

and enzymes [21]. RAAS is certainly involved in essential hypertension development [21]. ANP, an essential component of the RAAS, has been shown to be involved in smooth muscle contraction through cGMP kinase, diuresis, and inhibition of RAAS, affecting salt and water retention and blood pressure [37,38]. ANP gene was previously proved to encode high blood pressure levels in animal models [39].

In our study, we detected down-regulation of ANP gene expression in EH patients as compared to normotensive controls. We also noted a negative correlation between ANP gene expression and both SBP and DBP in EH patients. Therefore, we assumed that decreased expression level of ANP gene might be responsible for hypertension development among individuals. In concordance with our results, an earlier study on obese hypertensive patients revealed lower ANP gene expression levels compared to normal individuals [40]. Similar results were also obtained from previous studies carried out on animals showing decreased ANP mRNA and protein levels in hypertension [41]. A previous study showed similar results of low ANP gene expression of the mRNA in EH with the possibility of being a key factor in the disease progression [21].

Wang et al. demonstrated that obese individuals had low ANP serum levels and obesity is a well-known risk factor for hypertension [42]. A previous study on rats showed that gene expression of cardiac natriuretic peptide receptor-A was reduced on infusion of angiotensin II in hearts [43].

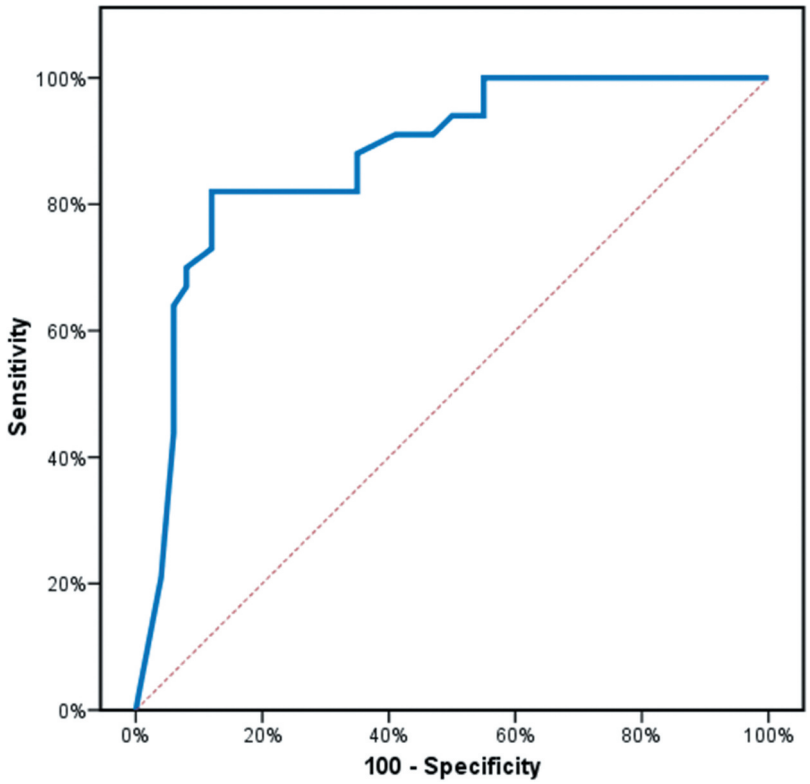

Figure 3. ROC curve for ANP gene expression to detect high (a) SBP and (b) DBP patients (vs control).

Table 3. Agreement (sensitivity, specificity) for ANP gene expression to detect high A) SBP and B) DBP patients (vs control).

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
ANP gene expression	0.874*	<0.001*	0.824–0.924	≤0.6	82.0	88.0	87.2	83.0

AUC: Area Under a Curve.
p value: Probability value.
CI: Confidence Intervals.
NPV: Negative predictive value.
PPV: Positive predictive value.
*: Statistically significant at p ≤ 0.05.

The RAAS inhibitors as ACE inhibitors and Ang II type 1 receptor blockers (ARBs), despite being effective in reducing blood pressure, they do not prevent end-organ damage. This led to the exploration of alternative treatments for hypertension. Ghatage et al. discussed recent developments in RAAS therapy that might treat hypertension as well as improve end-organ damage. They shed lights on several peptides and non-peptides with better effects in hypertension treatment that are still under research, for instance, angiotensinogen siRNA and AT2 receptor stimulators [2,44,45].

Another similar study conducted by Ghodsian and his colleagues studied ANP genetic analysis in EH. They screened genetic polymorphisms of ANP among the Malaysian population. They proved that ANP gene polymorphism was a risk factor for hypertension [46]. Another study showed that ACE genetic polymorphisms were independent risk factors for hypertension [47].

A recent study demonstrated that Ang II inhibited natriuretic peptide receptor 1 (Npr1) transcription by phosphorylating cAMP response element-binding protein (CREB) protein. This Npr1 gene transcription inhibition critically induced hypertension and cardiovascular dysfunction They recommended cotreatment with class I histone deacetylase (HDAC) inhibitors to reverse Ang II inhibitory effect on Npr1 expression [48].

Some limitations of our study should be mentioned. These included small sample size and follow-up of the patients to demonstrate the effect of ANP gene expression on hypertension over time and with treatment.

In conclusion, our study showed that ANP gene expression was low in essential hypertension patients compared with normotensive individuals and that new antihypertensive medications are required in the future in order to treat hypertension as well as its complications especially on the cardiovascular system.

Acknowledgments

We sincerely thank all patients and healthy volunteers for their participation in the current study.

Data availability

The datasets used during the current study are available from the corresponding author on reasonable request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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