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Correlation of Atrial Natriuretic peptide (ANP) coding gene (NPPA) and Human Hypertension

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Abstract Atrial natriuretic peptide (ANP) is a polypeptide hormone secreted by atrial muscle cells that has an endogenous antagonistic effect on the renin-angiotensin-aldosterone system (RAAS). It is believed to regulate salt and fluid balance and blood pressure. Blood samples (150) from males aged less than 20 to 55 years were collected from Al-Salam and Ibn-Sena Teaching Hospital in Mosul from 1/2/2022 to 1/9/2022. These samples 5ml that include healthy and previously blood hypertension-diagnosed patients were put into two tubes for hormonal and molecular study and divided into three classes control, which includes 50 samples from healthy individuals; hypertension without treatment patients 40 individuals and patients with treatment 60 individuals. Each class was divided into five groups according to their age. group (1) less than 20, group (2) 20-29, group (3) 30-39, group (4) 40-49 and group (5) 50-59 years. Serum ANP hormone concentration was measured, and the results revealed significant differences at $(p < 0.01)$ between patients and healthy control groups. Hormone concentration had decreased in the 1,2 group while increased in groups 3,4,5 of treated and untreated patients compared with control groups with different group concentrations ranging from 28.86-23.16 pg/ml, indicating the effect of aging on hormone concentration. Also, the results revealed a different hormone concentration in untreated patient's groups that ranged between 12.97-28 pg./ml while the concentration of treated patient's groups ranged between 12.97-33.36 pg/ml, these differences in hormone concentration in all groups revealed the effect interference of aging and treatments that had a direct effect on the hormone concentration. Besides, the Amplicon copy numbers of the ANP coding gene (Nppa gene), considered an essential indicator of gene activities utilizing the real-time technique, showed a significant increase in patient groups, especially group (5) of both treated and untreated, compared with control. These results concluded that this hormone had an essential correlation with hypertension.

Key Words Hypertension, Atrial natriuretic peptide, ANP, ANP gene, Nppa gene

1. Introduction

Atrial natriuretic peptide (ANP) is released primarily from the cardiac atria and cleaved extensively before conversion to its metabolically active form [1]. Human pre-pro ANP is a polypeptide with 151 amino acids (inactive form). The first 25 amino acids of pre-pro ANP are a signal sequence, which is proteolytically removed during processing, and a 126-residue, pro-ANP peptide is then generated and stored in secretory granules of atrial cardiomyocytes in atrial granules [2]. In the next phase, the pro-ANP is released into the blood circulation. It is sequentially proteolytically converted into two detectable plasmatic forms, ANP and NT-pro-ANP, by the atrial natriuretic peptide-converting enzyme (coring) [3], leading to a COOH-terminal biologically active peptide with 28 amino acids known as ANP [4].

Specific pathophysiology signals, such as atrial wall mechanical stretching, and several hormones (angiotensin II, catecholamines, or vasopressin) can promote the release of pro-ANP, which is rapidly cleaved by a transmembrane cardiac serine protease (Corin) producing the 28 amino acid, biologically active molecule ANP. Pro-ANP can also be alternatively cleaved by a different protease to urodilatin, a 32-amino acid peptide [5]. After secretion and cleavage, ANP enters the coronary sinus and is distributed to its target organs via circulation [4]. ANPs exert biological effects through interacting with two specific plasma membrane receptors: the principal receptor, or natriuretic peptide receptor A (NPRA), and natriuretic peptide receptor C (NPRC) [6]. NPRA is

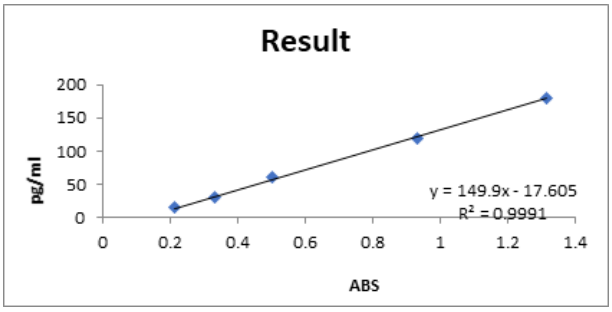

Figure 1: Relationship between concentration and optical absorbance in the ELISA device

commonly expressed in various tissues, including vasculature, heart, kidney, lungs, adrenal glands, adipose tissue, testis, liver, smooth muscle tissues, immune tissues, and some cancers. The expression of NPRC is also ubiquitous, including the heart, lung, adrenal gland, brain, liver, and immune tissues [7].

The human NPPA gene that encodes the ANP precursor is located on the short arm of chromosome 1 (1p36.21). This gene consists of 3 exons spanning more than 2 kb. The NPPB gene, encoding the BNP precursor, has a similar genomic structure and is located 10 kb upstream of the NPPA gene. Most likely, these genes were derived from gene duplication. In contrast, the human NPPC gene, encoding the CNP precursor, is on the long arm of chromosome 2 (2q37.1), indicating that it was segregated from the NPPA and NPPB genes during evolution [8]. This gene is located adjacent to another member of the natriuretic family of peptides on chromosome 1 [9], which produces protein belonging to the Neuropeptides group. It is length is 2515 bp, and the DNA in this gene is linear.

2. Material and Methods

Samples Collection

Venous blood samples (5 ml) from fasting male-only patients and healthy individuals were collected from Mosul's Al-Salam and Ibn-Sena teaching hospitals from 1/2/2022 to 1/9/2022. These samples were collected from 3 classes; Control includes 50 samples from healthy individuals, Hypertension without treatment patients 40 individuals, and patients with treatment 60 individuals. Each class was divided into five groups according to their age: group (1) less than 20, group(2) 20-29, group (3) 30-39, group(4) 40-49, and group (5) 50-59 years.

Samples were placed inside free anti-clotting gel test tubes, left at room temperature, and divided below 3ml. They were placed in a gel tube, centrifuged to obtain serum, and transferred to an Eppendorf tube. Moreover, 2ml was put in an EDTA tube for molecular study, and both Eppendorf and EDTA tubes were kept at -20°C. The concentration of the serum's ANP was estimated using an assay kit from a tube tech company of China origin, using the Enzyme-Linked Immunosorbent Assay technique (ELISA). Referring to the standard curve of the assay kit, the hormone concentration had been estimated Figure 1.

Extraction of DNA

Extraction of blood DNA of hypertensive and healthy individuals had been made utilizing the assay kit (AddPrep) processed by the Korean company Addbio [10].

Determination of DNA Concentration and Purity

The concentration and purity of DNA (Deoxyribonucleic acid) were quantitatively and qualitatively estimated using a device Implen Nanodrop Photometer Spectrophotometer.

Primers Design

Primers of Nppa gene had been Designed by (NCBI) the National Center for Biotechnology Information, Primer Blast website, as shown in the Table 1.

Q-PCR Amplification for DNA

Previously prepared, extracted DNA (25 microliters) had been utilized in the process of amplification using the primers for specific genes prepared by the American company "Promega," catalog no. 72050. As show in Table 2. The Eco Real Time PCR System uses heat-map to complete the reaction that shown in Table 3.

3. Statistical Analysis

Determination of any differences between control and patients (treated and un treated) classes had been made by Complete randomized design (C.R.D.) and Duncan's Multiple Range Test. Means, standard errors and the Significant difference at $(p \leq 0.01)$ probability level for each group had been estimated.

4. Results and Discussions

Serum ANP Concentration

Hypertension is a complex trait with multiple environmental and genetic Contributors (Newton-Cheh et al. [11]). It is a heritable trait, with heritability ranging from 15 to 40% for the clinic systolic blood pressure and from 15 to 30% for clinical diastolic blood pressure (Kupper et al. [12]). The risk of hypertension is significantly increased with one or two hypertensive parents (Chen and Wang [13]).

The results of serum ANP hormone concentration showed that there is a significant differences at a probability level ($p < 0.01$) between the two patient's classes treated and untreated compared with the control group as shown in Figure 2,

Hormone concentration had decreased in 1($<$ 20 years), 2 (20-29 years) groups while increasing in groups 3 (30-39 years), 4 (40-49 years), 5 (50-55 years) of treated and untreated patients compared with control groups that had different group's concentration ranged from 28.86-23.36 pg/ml which indicate the effect of aging on hormone concentration. Also, the results revealed a different hormone concentration

Gene	Primers sequence	Primers length	GC %
ANP Forward	5'- CTGGGAGACACCTTCAGCAG-3'	20 mer	60
ANP Revers	5'-CTGGGAGACACCTTCAGCAG-3'	20 mer	60

Table 1: Primers design

Constituent	Volume	Final Conc.
Master Mix PCR 2X	12.5 μl	1X
Upstream primer, 10 μM	0.25-2.5 μM	0.1-1.0μM
Downstream primer, 10 μM	0.25-2.5 μM	0.1-1.0μM
Template DNA	1-5 μM	<250ng
Free Water- Nuclease	25 μM	N.A

Table 2: PCR amplification for DNA process

Stage	Step	Temperature (C°)	Duration	Cycles
Polymerase Activation	Step1	95	00:02:15	1
PCR Cycling	Step1	95	00:00:15	40
PCR Cycling	Step2	60	00:01:00	40
PCR Cycling	Step3	60	00:01:00	40
Total Program Length	1 Hour 51 Minutes 37 Seconds			
Total Cycle Count	40			

Table 3: The heat map of Eco real time PCR system

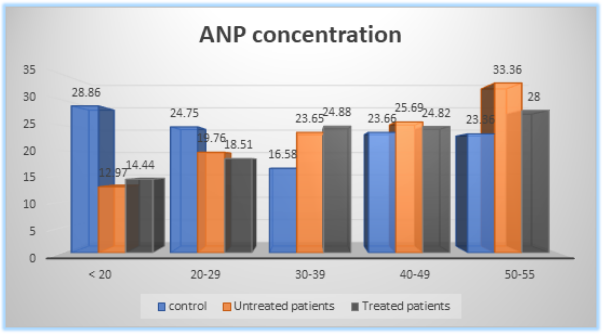

Figure 2: Comparison of serum ANP concentration between control and hypertension patients treated and untreated

in untreated patient's groups that ranged between 12.97-33.36 pg/ml while the concentration of treated patient's groups ranged between 14.44- 28 pg/ml. These differences in hormone concentration in all groups revealed the effect interference of aging and treatments that had a direct effect on the hormone concentration.

An increase in blood ANP hormone concentration prevents high blood pressure, while the decrease elevates blood pressure due to its physiological effects, including natriuresis, blood pressure regulation, and renin-angiotensin-aldosterone system (RAAS) antagonism. This research is consistent with [14], [15] results, which explain that cardiac natriuretic hormone concentration is lower in early adulthood (20- 40 years), especially in men.

The result of the G5 of untreated patients had high levels of serum ANP hormone compared with control but suffering from high blood pressure. This is due to another cause of blood pressure, such as kidney disease, undiagnosed heart disease, or due to advanced age, unless there is a deficiency in ANP hormone concentration.

There is a correlation between hormone concentration and patient's age in both untreated and treated groups, and this is consistent with the results of many researchers such as [14], [16]–[19] that all concluded that ANP synthesis is increased to combat the rise in pressure caused by different factors such as hardening of the arteries due to aging [19].

The level of this hormone was lowest in the untreated patient group. This may explain why these people suffer from high blood pressure, as the lack of secretion of ANP hormone leads to the retention of sodium and water in the body and increases the tension of blood vessels and blood pressure. If the ANP decreases, the cGMP level will fail to inhibit ENaC activity in the cortical collecting ducts, increasing sodium reabsorption and resulting in more pronounced hypertension [20].

The results show that an increase in ANP concentration in the G5 of the untreated and treated patients may be associated with changing atrial natriuretic peptide receptor density, possibly involving down-regulation [9].

The results also showed that there was a discrepancy between the concentrations of the hormone among patients with high blood pressure, whether they were treated or untreated. This discrepancy may be attributed to the antihypertensive drugs that work in several ways, including what affects the secretion of ANP or affects the RAAS pathway (Ma et al. [21]).

Another reason for the high concentration of ANP in the serum may be due to the beginning defect of the kidney's work, which leads to a decrease in the process of getting rid of sodium ions and water, which leads to an increase in blood volume and thus an increase in the secretion of the hormone [22], ANP elevated in the majority of patients with kidney disease due to reduced renal excretion, these levels still demonstrated associations with volume overload, abnormal cardiac structure and function, cardiovascular diseases,

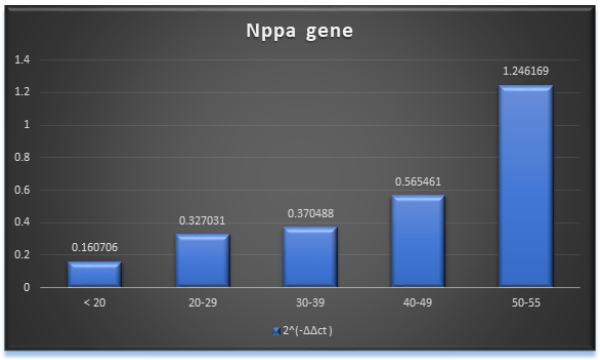

Figure 3: The value of $2^{(}-\Delta\Delta ct)$ of the Nppa gene explain the comparison of Amplicon copy numbers of ANP coding gene (Nppa gene) between Healthy control and hypertension patient's groups

cardiac events and mortality [23].

The increase in the serum hormone concentration of all individuals treated and untreated may be attributed to their lifestyle, as the increase of sodium salts intake in food leads to an increase in the secretion of the hormone [24]. Salt intake, elevated extracellular fluid osmolality, enhanced blood volume, rapid heart rate, and neurohumoral factors directly affect the pressure on the atrial muscle and the release of ANP. Serum sodium levels can stimulate ANP that affects BP regulation [25].

ANP concentrations noted to occur in those with a controlled SBP in relationship to increases in volume load (as evidenced by renal indexes of fluid retention and increases in systemic flow) are attenuated in those with an uncontrolled SBP and markedly higher indexes of volume load [26].

The results showed that most ANP forms tend to decrease or are significantly decreased in early hypertension and then tend to increase only in late hypertension. These findings confirm the relative deficiency of such a protective hormonal system, possibly due to an impaired release and/or an elevated metabolism of the mature and biologically active forms of these cardiac hormones; these results agree with the result of [27].

DNA concentration between different groups ranged between (18- 68 ng) and the purity was ranged between (1.8-2) which indicated the high purity of DNA samples of all groups. Besides the results revealed that amplicon copy numbers of ANP coding gene (Nppa) had been increased in all patient's groups treated and untreated compared with control and housekeeping gene, as shown in Figure 3.

This gives an indirect indication of the expression of this hormone. However, this increase did not lead to an increase in the serum hormone concentration in patients 1,2 groups treated and untreated compared with control groups, but there was a decrease in the concentration in these serum groups due to many reasons; one of the themes is post-translational modification who converts the pro-ANP hormone to active one. Another reason may be the mutations that occurred in this gene [28], found several single nucleotide polymorphisms (SNPs) in this gene. One of them (-C664G) is located in the promoter region, which is associated with low plasma ANP levels and high blood pressure in patients. This mutation abolished the stop codon, creating a mutant molecule with 12 extra amino acids at the C-terminus of ANP. Another SNP is rs5068, which lies in the 3'-UTR of the NPPA gene and encodes the pro-peptide of ANP, NT-pro ANP. The risk of the allele rs5068 showed lower NT-proANP expression, possibly mediated through a microRNA, and reduces ANP level and consequently increases Blood pressure [29].

Another reason may be a mutation in the ANP receptor coding gene [30], was identified as an 8-bp deletion in the 5 -5-flanking regions of the ANP receptor gene. This mutation impaired the promoter activity when tested in vitro and was associated with hypertension and cardiac hypertrophy. DNA methylation levels at NPPA promoter may cause hypertension [31], found that the hypermethylation at NPPA promoter is associated with a lower level of blood pressure and a lower risk of having hypertension.

Mutations can occur in the human Corin gene [32], identified Two non-synonymous SNPs (T555I/Q568P) in a minor Corin allele which associated with an increased risk for hypertension and an enhanced cardiac hypertrophic response to high blood pressure response to dietary salt [33]. Corin deficiency may contribute to hypertension in humans and alter patients' response to antihypertensive drug treatment [34].

ANP deficiency due to expression deficiency of Nppa led to lower production of cGMP. The decreased cGMP levels failed to inhibit ENaC activity in the cortical collecting duct, which increased sodium reabsorption and resulted in more pronounced hypertension [20], [35].

5. Conclusion

The results of this research revealed that BP is a complicated case that may interfere with many factors, aging is the important one that has an important role in BP elevation due to the defect in the expression of many genes such as receptors of hormone and many enzymes that have an important role in the RAAS pathway. Beside Mutations can be occurred in many human genes such as ANP hormone or corine enzyme coding genes.

Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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