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The Physiological and Molecular Evaluation of BNP and Its Effect on Natriuretic Peptide Levels in Patients with Congestive Heart Failure

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Abstract

Objective: The current study was conducted to determine the relationship between BNP gene polymorphisms and some hormonal variables of a group of congestive heart failure patients.

Materials and Methods: The study comprised 60 people with congestive heart failure and 30 people who were healthy. The Enzyme Linked Immunosorbent Assay (ELISA) was used to detect serum hormone levels, and the Tetra ARMS-PCR technique was used to genotype the BNP gene for locus rs198389.

Results: The study's findings revealed a highly significant decrease in Atrial Natriuretic Peptide (ANP) concentration, with an average of (29.879± 6.9864) and (34.074 ±5.7455) pg/ml, and a highly significant increase in the concentration of Brain Natriuretic Peptide (BNP) with an average of (123.3839 ± 5.900) and (96,583 ± 6.191) pg/ml, and a highly significant decrease in the concentration of C-type natriuretic peptide (CNP) with an average of (174.402 ± 8.943) and (261.651 ± 9.172) pg/ml respectively at the level of probability ($P \leq 0.01$) in serum Congestive heart failure as compared with those in the control group.The results of the polymorphism of the brain natriuretic peptide gene for the rs198389 locus revealed that there were differences in the frequency of alleles and genotypes in the patient group compared to the control group, but these changes were not statistically significant. The mutant GG genotype was found in a high number of patients (OR = 2.6) compared to controls, and it was dominant over the normal genotype, and with OR = 1.350 for the G mutant allele. Thus, it is considered as a risk factor for the disease. The mutant genotype was associated with highly significant decrease in the levels of Atrial Natriuretic Peptide (ANP) and a highly significant increase of both Brain Natriuretic Peptide (BNP) and C-type Natriuretic Peptide (CNP) at the level of probability ($P \leq 0.01$) in the group of patients with congestive heart failure.

Conclusion: It can be concluded that low levels of ANP and CNP, as well as the existence of the G allele of the BNP gene for the rs198389 locus in congestive heart failure patients, play an important role in illness development and patient therapy.

Introduction

Due to its high incidence and mortality, congestive heart failure (CHF) is the most common cardiovascular disease and has become a rising global issue with an aging population (Glava, 2020). Heart failure affects around 64.3 million people globally (James et al., 2018). Congestive

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heart failure is caused by structural and/or functional alterations in the heart, which result in weak ventricular systole or diastole and, as a result, a decrease in cardiac output and/or intracardiac pressures at rest and during stress (Salzan et al., 2021; Hsu et al., 2021). It is either sudden onset, such as myocardial infraction, or gradual, such as high blood pressure, or it may be genetic, regardless of the pathogenesis, and the result is a decrease in heart function (Lainscak, 2017).

Three peptides with similar structures but different characteristics are produced by the heart, which functions as an endocrine organ. The transmembrane receptors of the quanyl cyclase are the means by which these peptides—atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP)—perform their respective biological activities. (Ramakrishnan and others, 2020). DeBold and his team found a protective hormone called ANP in 1981. (De Bold et al., 1981; Ichiki et al., 2017). With excessive levels of ANP in the ventricles and blood plasma associated with cardiac disorders like hypertension, hypertrophy, and heart failure, the atria are the primary source of ANP production (Lee et al., 2020). First found in the pig brain in 1988, BNP is now known to be produced by ventricular cardiomyocytes and stored in secretory vesicles (Zhou et al., 2018; Hal, 2004). According to Ma et al. (2021; Jeson Sangaralingham et al., 2011), endothelial cells, cardiomyocytes, and fibroblasts create the majority of CNP in the kidneys (Sangaralingham et al., 2020; Moyes and Hobbs., 2019)

Both ANP and BNP work to counteract the effects of angiotensin II through salt and diuresis, vasodilation, and aldosterone inhibition (Kuwahara, 2021). It has significant, bidirectional effects on controlling blood pressure, reducing ischemia, angiogenic factor, anti-inflammatory, and anti-atherosclerotic processes in the vascular system (Forte et al., 2019). Important features in heart failure include renin-angiotensin, which blocks endothelin, vasopressin, improves cardiac epinephrine regulation, and vasodilatation (Del Ry, 2013).

Changes in the DNA sequence, such and single nucleotide polymorphisms, occur more frequently than 1% of the mutations (Sukhumsirichart, 2019). The human genome is where it most frequently happens, and it is described as a change in a single base or single base of a DNA sequence (Sukhumsirichart, 2019).

The gene NPPB, which produces BNP, is found on the short arm of chromosome 1 ($1_P36.2$) of the human genome and has three exons and two introns (Kleine and Rossmanith, 2016). The untranslated region at end 5 , part of preproBNP 16 amino acid and the first 18 amino acids of ProBNP are encoded by the first exon of the BNP gene, while the amino acids from 129 to 54 are encoded by the second exon and amino acids from 134 to 130 are encoded by the third exon and the untranslated region at term 3 by the third exon, respectively (Al-Ibrahimi et al ., 2016; Sudoh et al., 1989).

The natriuretic peptide system genes contain a number of single nucleotide polymorphisms (SNPs), which are crucial for the aetiology, therapeutic use, and development of cardiovascular disease (Vassalle and Andreassi, 2009).

The purpose of this study was to investigate the relationship between a group of congestive heart failure patients' hormonal characteristics and BNP gene polymorphisms.

Materials and Methods

Study Group

Samples were taken between the beginning of November 2021 and the end of April 2022. (90) samples were used in the investigation. It included 60 patients (33 men, 27 women) ranging in

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age from 50 to 80 years, and 30 healthy adults (16 men, 14 women) ranging in age from 50 to 65 years.

Sample Collection

5 ml of venous blood (from both patients and healthy) was collected and deposited in test tubes with Gel. It was placed in a 37° C water bath for 10 to 15 minutes, until the blood clotted. Then it was separated by centrifugation at 3500 cycles per minute. It takes 10 minutes to obtain a blood serum.

Testing

The concentration of CNP, BNP, and ANP peptides in the serum of congestive heart failure patients and healthy subjects was determined according to the instructions of the Enzyme Linked Immunosorbent Assay (ELISA) from Sunlong Biotech, China.

Molecular tests of BNP genotyping

DNA was extracted from white blood cells using coulombs (separation columns) in various steps for the patient and control groups using the method described by (Yuda and Saputra, 2020). DNA samples were stored at (-80 degrees Celsius). The Tetra-Amplification Refractory Mutation method was employed. Tetra-ARMS-PCR system for detecting the BNP gene polymorphism at the rs198389 locus. This study used four primers, as shown in Table 1. The additions were carried out with the used of a premix kit, followed by 15µl of deionized distilled water, 3µl of Primers, 2µl of DNA sample, and the reaction was carried out with the help of the thermopolymer programme, as given in Table (2).

Table (1) Brain Natriuretic Peptide gene sequences

NO	Primers	Sequence
1	Inner Forword	GCTTCTTCCTTTCCTGCAAATGTACA
2	Inner Reverse	AAAGCGCCAACCTAGGACAACC
3	Outer Forword	CTGCCATGCAGGGTTATCTCTGAT
4	Outer Revers	GGGGCTGTTTTCGCTGTGAGT

Table (2) Thermopolymerization program for the Brain Natriuretic Peptide gene

Stag	Temperature	time seconds/minute	number of rounds
Pre Denaturation	95	4 minutes	1
Denaturation	95	30 seconds	35
Anneling	62	45 seconds	
Elongation	72	45 seconds	
Terminal elongation	72	5 minutes	1

Statistical Analysis

The results of the hormonal tests were analysed using the Student's t-test and the Anova Test for the group of patients and healthy subjects at a significant level of($p \leq 0.01, p \leq 0.05$), then according to the allelic frequency of the (BNP) gene and the number and percentages of homologous, heterologous, and mutated genotypes for the group of patients. The Odd Ratho (OR) value of alleles and genotypes in the group of patients and healthy people was also calculated.

Results

According to the findings provided in Table3, the current study found a highly significant decrease in the concentrations of ANP and CNP in the blood serum of congestive heart failure patients compared to the control group ($p \leq 0.01$) with a mean of (29.879 ± 6.9864) and (34.074 ± 5.7455), and with a mean of (174.402 ± 8.943) and (261.651 ± 9.172) pg/ml, respectively. And a highly significant increase in the concentration of BNP in the blood serum of patients with congestive heart failure compared with the control group at a significant level ($p \leq 0.01$), according to the values shown in Table (3), with an average of (123.3839 ± 5.900) and (96,583 ± 6.191) pg/ml, respectively.

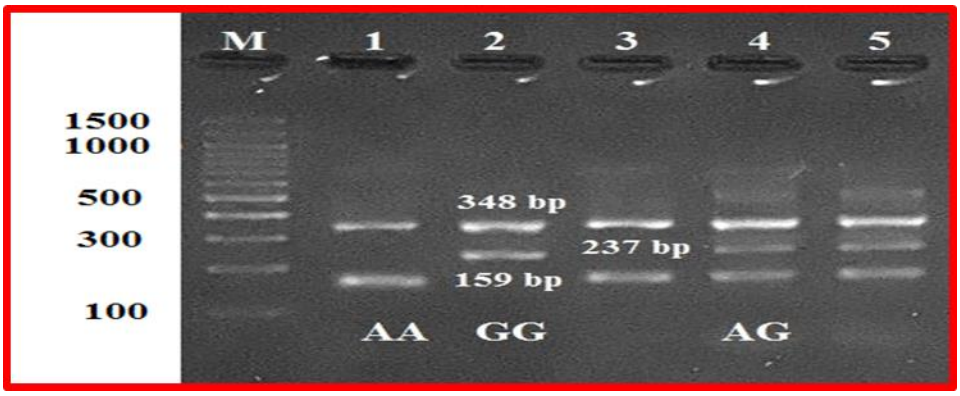
Table 3. Shows comparison between Natriuretic peptide levels of the Patients and Control.

Parameters	Patients (No. 60) Mean ± SD	Control (No. 30) Mean ± SD	*P* value
ANP pg/ml	29.879 ± 6.9864	34.074 ± 5.7455	≤ 0.01 **
BNP pg/ml	123.3839 ± 5.900	96.583 ± 6.191	≤ 0.01 **
CNP pg/ml	174.402 ± 8.943	261.651 ± 9.172	≤ 0.01 **

non significant,p>0.05, *Significant, p<0.05, ** High significant, p<0.01.

The brain natriuretic peptide gene polymorphism of the rs198389 locus

The results of the Tetra-ARMS-PCR analysis of the BNP gene for the rs198389 locus showed three genotypes (AA, AG, and GG) as shown in Picture (1) .



Picture (1) Electrophoresis of the results of the amplified segment of the BNP gene using Tetra-ARMS-PCR technique, on a 2% agarose gel.

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Figure (1) depicted three genotypes: the normal AA genotype represented by two bundles (348bp, 159bp), the mutant GG genotype represented by 348bp and 237bp bundles, and the heterozygous AG genotype represented by three bundles (348bp, 237 bp,159bp).

Percentage, genotypes, and frequency of alleles for the BNP gene for locus rs198389 for the patient and control group.

The study's findings revealed the BNP gene at rs198389, as shown in Table (4). The patients had the highest percentage of subjects with the heterozygous AG genotype (61.67%) compared to the control group (70%). It reached (16.66%) in the patients group compared to (20%) in the control group for the normal genotype AA.

The results also revealed that the percentage of patients with the normal allele A was 47.5 percent compared to 55% for the control group, and that the percentage of patients with the mutant allele G was 52.5 percent compared to 45% for the control group.

When comparing the patient group and the control group, the risk factor value was OR = 1.350 with a 95% confidence interval (CI) of 0.7251-2.516 in the ratio of the frequency of the mutant allele G to the normal allele A. This suggests that the mutant allele G very mildly increases the probability of developing the condition.

Table (4): Percentage, genotypes, and frequency of alleles for the BNP gene for locus rs198389 for the group of patients and control.

Genotypes	Patients No. (60)		Control No. (30)		OR	(95% CI)	*P* value
	No.	%	No.	%			
AA	10	16.66	6	20	1 Ref.	-	-
AG	37	61.67	21	70	1.057	0.3364 - 3.322	0.924
GG	13	21.67	3	10	**2.6**	0.5184 - 13.041	0.245
Alleles	**No.**	**%**	**No.**	**%**	**OR**	**(95% CI)**	***P* value**
A	57	47.5	33	55	1 Ref.	-	0.342
G	63	52.5	27	45	**1.350**	0.7251 to 2.516	

Concentration of natriuretic peptides by genotypes of the BNP gene at locus rs198389 in patients with congestive heart failure

The findings of the current study are displayed in Table (5). When GG genotype was compared to AA genotype, with a mean of (27.730±2.432) versus (27.891±4.924), there was a highly significant drop (P ≤0.01) in the concentration of ANP. Additionally, there was a highly significant rise in BNP and CNP concentration for the GG genotype at (P≤0.01), with average values of (115,514±6.730) and (218.028 38.536), respectively, as opposed to the genotype AA, with average values of (114.375± 4.991) and (208.941 ±8.473). According to Figure (1).

Table (5) Concentration of natriuretic peptides by genotypes of the BNP gene at locus rs198389 in patients with congestive heart failure.

Parameter	AA (No. 10) Mean ± SD	AG (No. 37) Mean ± SD	GG (No. 13) Mean ± SD	*P* value
ANP pg/ml	27.891 ± 4.924	32.036 ± 5.438	27.730 ± 2.432	≤ 0.01 **
BNP pg/ml	114.375 ± 4.991	130.707 ± 5.467	115.514 ± 6.730	≤ 0.01 **
CNP pg/ml	208.941 ±8.473	150.550 ±23.444	218.028 ± 38.536	≤ 0.01 **

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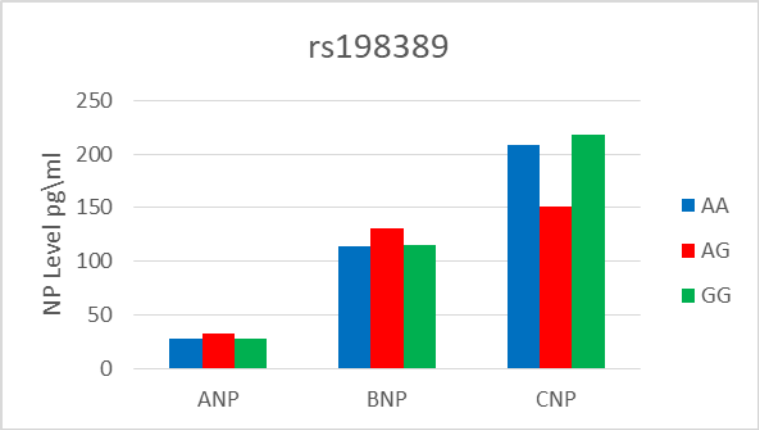
Figure (1) Concentration of natriuretic peptides by genotype of BNP gene at locus rs198389 in patients with congestive heart failure.



Discussion

Concentrations of CNP, BNP, and ANP in patients with congestive heart failure

The current study's findings are consistent with those of Matsuda et al. (2022), who found that repeated hospital admissions in patients with heart failure and remodeling of the left ventricle in those with atrial fibrillation resulted in a decrease in the ratio of atrial natriuretic peptide to the ratio of brain natriuretic peptide. It also agreed with Han et al. (2017) and Al-Tikriti (2021) found that the concentration of brain natriuretic peptide in the blood serum of the group of patients with congestive heart failure was significantly higher than that of the "healthy" control group.

Both ANP and BNP function as a protective mechanism against ventricular stress, the negative effects of volume and pressure overload, modifying pulmonary arterial blood pressure, and cardiac hypertrophy and fibrosis (Kuhn, 2016; Vinnakota and Chen.,2020). To combat the negative consequences of heart failure, it eliminates sodium, diuretics, dilates blood vessels by relaxing the smooth muscles of the arteries, maintains cardiac and renal balance by suppressing the sympathetic nervous system, and inhibits the renin-angiotensin-aldosterone system (Fu et al. ,2018). When strain and stress are intense, it is released from the ventricles, increasing the pressure on the heart in people with congestive heart failure (Gray, 2006).

The results of this study supported those of Kalra et al. (2010), who found that CNP levels were lower in congestive heart failure patients than in those with normal left ventricular function. This may be because CNP is produced and eliminated into the bloodstream by healthy kidneys, and renal secretion is reduced in people with congestive heart failure. This peptide's net effect on congestive heart failure patients. Low levels of CNP and a weakened endothelium lining are most likely the cause of this, where CNP is derived from the endothelial endothelium and any abnormality in the endothelium will lead to a decrease in the level of CNP and the occurrence of an imbalance in the blood vessels such as high-pressure Blood, arteriosclerosis, and thus heart failure (Moyes et al., 2014).

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The rs198389 locus BNP gene polymorphism in congestive heart failure patients and the control group

The findings of the present study were consistent with those of Al-Ibrahimi et al. (2016), who genotyped the BNP gene in the rs198389 (T-381-C) region in a group of heart failure patients. They found three genotypes, with the TC heterozygous genotype being the most prevalent, followed by the mutant genotype CC and the normal TT genotype. The ratio of the frequency of the mutant allele C to the normal allele A was, respectively, 42.9% and 34.0%, which suggests that the mutant allele C may be a risk factor for the disease.

The proportion of the mutant GG genotype was significantly higher in the group of COPD patients with pulmonary hypertension compared to the group of COPD patients without pulmonary hypertension at a probability level ($P \leq 0.01$), according to the results of genotyping in the rs198389 region of the BNP gene in a group of COPD patients. This suggests that the mutant G allele in the rs198389 region represents a significant risk factor for COPD with high pulmonary pres- (Jin et al., 2018). A polymorphism in the BNP gene's rs198389 area was discovered to be a potential genetic factor impacting the advancement of left ventricular dysfunction in people with coronary heart disease and dyslipidemia in a study on the people of southern China (Antoniuk et al., 2020). Patients with hypertension and heart failure in central and western Ukraine were more likely to have the mutant C allele than patients with the healthy T281T genotype. Greater ventricular wall thickness was linked to the normal allele. Myocardial mass index is much larger in mutant allele carriers than in non-carriers. (Pashkoval, 2016).

Concentration of natriuretic peptides by genotype of BNP gene at locus rs198389 in patients with congestive heart failure.

The findings of the current study support those of Xhaard et al. (2022), who found an association between the mutant allele at locus rs198389 and higher levels of NT-ProBNP. Additionally, BNP levels were considerably higher in those with the CC mutant genotype compared to those with the normal TT genotype, indicating that the C mutant allele in the T381C region is associated with elevated BNP levels in hypertensive individuals with LVH (Pashkova et al., 2015). In a group of healthy Russians, those with the CC genotype had higher BNP levels than those with the normal TT genotype, hence both the C allele and the CC genotype are thought to be protective factors against the risk and severity of heart failure (Berezikova et al., 2013). Higher amounts of BNP and NT-ProBNP are produced as a result of the mutant allele's effect on the inducer binding activity of the BNP gene (Johansson et al., 2016). The effects of BNP are to reduce the load on the heart by enhancing urine excretion and increasing capillary permeability, thus decreasing pressure (Goetze et al., 2020) and inhibition of fibroblast growth and collagen production (Gorący et al., 2015).

Despite the small number of mutant samples, the results of this study showed that each mutant G allele of the BNP gene at the rs198389 locus resulted in an increase in BNP levels and a decrease in ANP levels in serum, indicating that the genetic factor plays a role in cases of congestive heart failure.

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