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Elevated IL-6 and NT-proBNP in Heart Failure: Assessing the Diagnostic Potential of NKX2-5 Gene Expression

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ABSTRACT

INTRODUCTION: *Heart failure (HF) is a complex condition with inadequate cardiac output and elevated intracardiac pressures, prevalent among older populations worldwide. Chronic inflammation, marked by elevated cytokines like IL-6, and biomarkers such as NT-proBNP, are closely linked to heart function. The transcription factor NKX2-5, essential for heart development and maintenance, interacts with IL-6 and NT-proBNP, suggesting a role in inflammation and cardiac stress.**AIM**: The present study investigates the relationship between NT-proBNP, IL-6, and NKX2-5 in patients with heart failure.* ***MATERIALS AND METHODS****: The study included 160 participants divided into two groups: Group I and Group II. Venous blood samples were centrifuged and stored at -20°C for analysis. Biochemical investigations included measurements of FBS, cholesterol, triglycerides, HDL, LDL, T3, T4, TSH, RNA isolation, cDNA synthesis, NKX2-5 gene expression, and ELISA assays for IL-6 and NT-proBNP. Statistical analyses encompassed descriptive statistics, t-tests, chi-square tests, logistic regression, ROC curve analysis, and Pearson correlation coefficients, among other methods.* ***RESULTS****: The test and control groups exhibit significant differences in demographics and health factors. Genetic studies reveal reduced expression of the NKX2-5 gene in heart failure cases, potentially increasing the risk of developing the condition. Highly predictive biomarkers for heart failure include NT-*

proBNP and IL-6. Additionally, diabetes, dyslipidemia, and elevated IL-6 levels are identified as significant

risk factors. Correlations between these biomarkers and physiological parameters suggest complex interactions underlying heart failure.

***CONCLUSION**: The study found elevated IL-6 and NT-proBNP levels in heart failure patients, indicating increased inflammation and cardiac stress. An inverse correlation between NKX2-5 expression and these biomarkers suggests NKX2-5's potential role in regulating inflammation and cardiac function in heart failure.*

***KEYWORDS**: Heart Failure, Inflammation, Gene Expression, Nkx2-5 Gene, Il6, Ntprobnp, Cardiovascular Risk Factor*

INTRODUCTION

Heart failure (HF) is a clinical condition made up of cardinal symptoms that may or may not be accompanied by indicators rather than a single medical diagnosis. It is caused by an anomaly in the structure or function of the heart that leads to high intracardiac pressure and/or insufficient cardiac output while at rest or when exercising (1). HF is a global pandemic impacting 64 million people, with projections indicating a rise in prevalence due to aging of population. In the US, HF prevalence is expected to surge by 46% by 2030, leading to a 127% increase in healthcare costs (2). It is estimated that between 1.3 and 4.6 million people in India suffer from heart failure as a result of coronary heart disease, hypertension, obesity, diabetes, and rheumatic heart disease (3). About half of the cases from the diseases listed above entail heart failure with a preserved ejection fraction (HFpEF). HF is increasingly becoming a global concern, especially among older individuals and those residing in regions with low-to-medium socio-demographic index (SDI), leading to a continuous rise in prevalence and health burden (4). A crucial mechanism in the pathogenesis of HF is inflammation. In people with HF, higher levels of pro-inflammatory cytokines lead to worse outcomes and unfavorable cardiac remodeling (5).

It has been demonstrated that heart failure (HF) results in an elevation of inflammatory cytokines due to its chronic inflammatory condition, which has been linked with adverse consequences and cardiac remodelling (6). ln order to better comprehend how the development of future HFpEF vs HFrEF is associated with cardiovascular biomarkers that reflect many physiologic mechanisms, such as renal function, myocyte stretch, stress, and inflammation (7).

Biomarkers such as N-terminal pro b-type natriuretic peptide (NT-proBNP) and interleukin-6 (IL-6) have been associated with the pathophysiology of heart failure.

For cardiac development and adult heart function, the homeobox transcription factor Nkx2.5 is necessary. It recognizes and binds specific DNA sequences (8). NKX2.5 activates transcription of target genes (9). The nuclear location signal (NLS) is located within or near the homeodomain sequence, which facilitates the transport of NKX2-5 into the nucleus, enabling transcriptional regulation(10). During development NKX2-5 regulates the cardiac cell fate determination (11) and plays a very important role in heart chamber development (12). Nkx2-5 is also required for specification of the ventricular conduction system (13). More over, Nkx2.5 perform cardiac cell differentiation (14). Nkx2-5 regulates cardiac cell identity and function and helps to maintain cardiac output and heart function in adult (15). Heterotypic interaction between the NKX2-5 and other cardiogenic transcription factor genes influence atrial rhythm control (!6). NKX2-5 help to promote regeneration, and enhance overall heart repair mechanisms (17). The current study aims to explore the relationship between NT-pro BNP and IL-6 levels and NKX2-5 gene expression in individuals with heart failure.

MATERIALS AND METHODS

Study Design and Participants

This **case-control study** involved 160 participants, comprising 80 cases and 80 controls, all aged between 30

and 65 years. The Institutional Ethics Committee granted permission for the study to be carried out, and letter number Reg No. EC/NEW/INST/2022/2847]. Participants were recruited from a teritary care hospital, Thiruvananthapuram and genetic analysis was performed in **Genetika, Centre for Advanced Genetic Studies,** Thiruvananthapuram, Kerala. Informed consent was obtained from all subjects p**rior to sample collection.**

Inclusion and Exclusion Criteria

The study population included 160 individuals divided into two groups. The case group (Group I) consisted of 80 patients confirmed with heart failure. The control group (Group II) consisted of 80 age- and sex-matched healthy subjects.

Sample Collection

The sample comprised 160 clinically proven patients aged 30 to 65 years, chosen according to the inclusion and exclusion standards. Each participant gave a total of 5ml of fasting venous blood. The blood samples were centrifuged, and collected serum was kept for further analysis at -20°C. Data was collected using a **pre-structured questionnaire**, which included general and anthropometric data (weight, height), age, and onset of symptoms. The ratio of weight (kg) to squared height (m^2) was used to determine the body mass index (BMI).

Biochemical Analysis

Biochemical Markers Detection

To assess fasting blood sugar (FBS), an automated biochemical analyzer was utilized, while cholesterol, triglycerides, HDL, and LDL levels were assessed using **enzymatic colorimetric methods**. Thyroid Hormones DetectionThyroid hormones, including triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH), were measured using **chemiluminescent immunoassay (CLIA)**.

RNA Isolation and cDNA Synthesis

For genetic analysis, RNA isolation was performed from blood samples using a commercially available RNA extraction kit (spin column). Complementary DNA (cDNA) synthesis was conducted using reverse transcription. Total RNA (50 ng) was reverse transcribed using the Origin cDNA synthesis kit (CAT.NO. ODP419)with an RNase inhibitor, according to the manufacturer's guidelines

.

Gene Expression

The gene expression level of the NKX2-5 gene was assessed using 2X Real-Time PCR Master Mix (including SYBR Green): 4× 1 mL. The temperature and time programmes, and protocol for expression analysis, were performed with the Bio-Rad CFX-Opus-96 machine and Bio-Rad CFX Maestro software. Specific primers (F: 5'-AAGTGTGCGTCTGCCTTTCCCG3', R: 5'-TTGTCCGCCTCTGTCTTCTCCA-3') for NKX2-5 gene expression were provided by Eurofins Genomics India Pvt.Ltd. Relative NKX2-5 expression was computed by applying the 2^(-ΔΔCt) technique.

Enzyme-Linked Immunosorbent Assay (ELISA)

Antibody-coated ELISA kits were used for detecting IL-6(CAT.NO.opk1156), and NTproBNP(CAT.NO.OPK1212) in human blood serum. The quantitative sandwich enzyme immunoassay technique was employed to measure the levels of these biomarkers. Samples were tested according to the ELISA kit(Origin Diagnosis), with temperature and time programmes for the assay examined by the Thermo Scientific Multiskan F.C. machine and Skanit RE 6.1.1 software.

STATISTICAL ANALYSIS

The statistical procedures employed in this study involved a comprehensive analysis of various demographic, lifestyle, and health-related factors associated with heart failure. Initially, descriptive statistics were calculated to summarize the attributes of the study group (N = 80) and control group (N = 80), including of categorical variable frequencies and percentages and continuous variable means with standard deviations. For continuous variables, independent sample t-tests were used, and for categorical variables, chi-square tests were used to evaluate. To assess differences between the two groups, independent sample t-tests were utilized for continuous variables, while chi-square tests were applied for categorical variables differences between the two groups. $P < 0.05$ was designated as the significant level. The association between several predictors and the chance of heart failure was also examined using binary logistic regression analysis, which allowed for the correction of possible confounding variables. For each predictor, odds ratios (OR) with 95% confidence intervals (CI) were computed to measure the strength of relationships. A Receiver Operating Characteristic (ROC) curve analysis was also performed to evaluate the diagnostic accuracy of NT-proBNP as a predictor of heart failure, Furthermore, Pearson correlation coefficients were computed to explore the relationships between inflammatory markers, NT-proBNP, and other biochemical parameters. The statistical analyses were performed using appropriate software, ensuring robust and reliable results that contribute to the understanding of heart failure risk factors. Data analysis was performed using "JAMOVI 2.5.3".

RESULT

The findings indicated a significant association ($p < 0.05$) in demographic variable measurements between the test and control groups, as shown in the Table 1. The average age of the experimental group was 49.8 ± 5.2 years, whereas the control group's average age was 49.1 ± 7.6 years (p=0.490). This suggests that age is similar across both groups. Conversely 50% of the study group were male versus 36.2% in the control group. The case group exhibited notably higher levels of abdominal circumference, weight, BMI, fasting blood sugar, serum triglycerides, total cholesterol, LDL cholesterol, T3, T4, TSH, IL-6, NT-proBNP, and significantly lower levels of HDL cholesterol and NKX2-5 gene expression in contrast to the control group. These differences implies that the study group has a higher risk profile for heart failure.

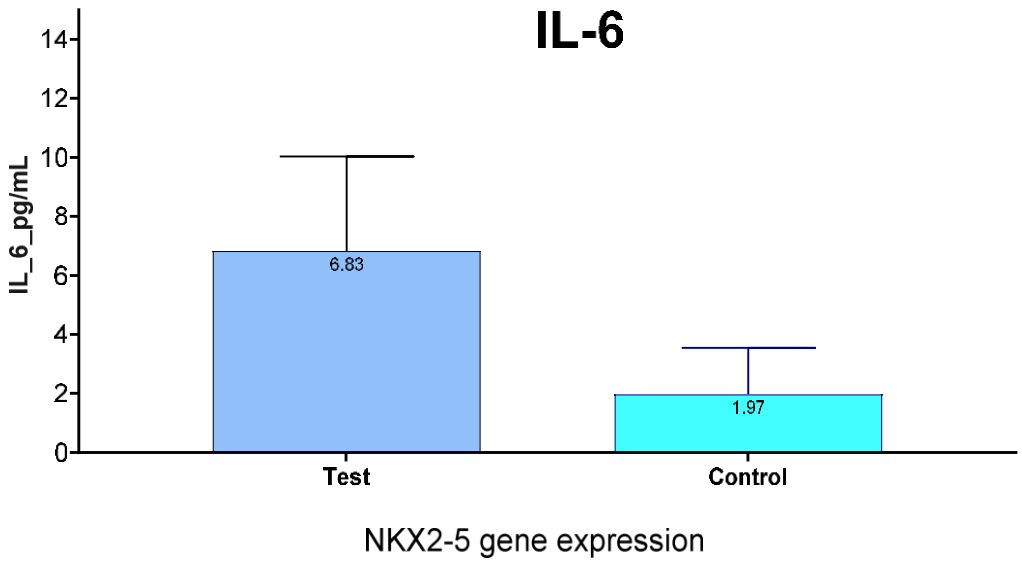
	case (n=80)	Control (n=80)	P value
Age	49.8 ± 5.2	49.1 ± 7.6	0.490
Duration of Married life in years	24.5 ± 8.5	23.5 ± 7.9	0.448
Age at Marriage	25.6 ± 6.6	25.3 ± 4.7	0.738
Abdominal Circumference in cm	89.6 ± 19	72 ± 15.2	< 0.001
Height in cm	157.9 ± 10.8	159.3 ± 10	0.397
Weight in Kg	68.1 ± 13.1	59.2 ± 9.5	<0 .001
BMI	27.4 ± 4.9	23.2 ± 1.7	< 0.001
Fasting Blood Sugar mg/dL	145.6 ± 35	92.5 ± 13.8	< 0.001
Serum Triglyceride mg/dL	164.1 ± 38.4	105.4 ± 21.5	< 0.001
Serum Total Cholesterol mg/dL	241 ± 41.4	174.1 ± 26	< 0.001
Serum HDL Cholesterol mg/dL	37.8 ± 6.4	44.4 ± 4.9	< 0.001
Serum LDL Cholesterol mg/dL	170.4 ± 34.8	108.6 ± 23.5	< 0.001
T3 pmol/L	72.8 ± 16.4	120.8 ± 23.6	<0.001
T4 nmol/L	4.13 ± 1.01	7.59 ± 1.93	< 0.001
TSH_µIU/mL	7.16 ± 4.07	2.36 ± 0.98	< 0.001

IL_6_pg/mL	6.83 ± 3.2	1.97 ± 1.58	< 0.001
NTproBNP_pg/mL	135.8 ± 41.3	73.2 ± 32.2	< 0.001
NKX25_gene_expression	0.79 ± 0.612	1.021 ± 0.573	0.015

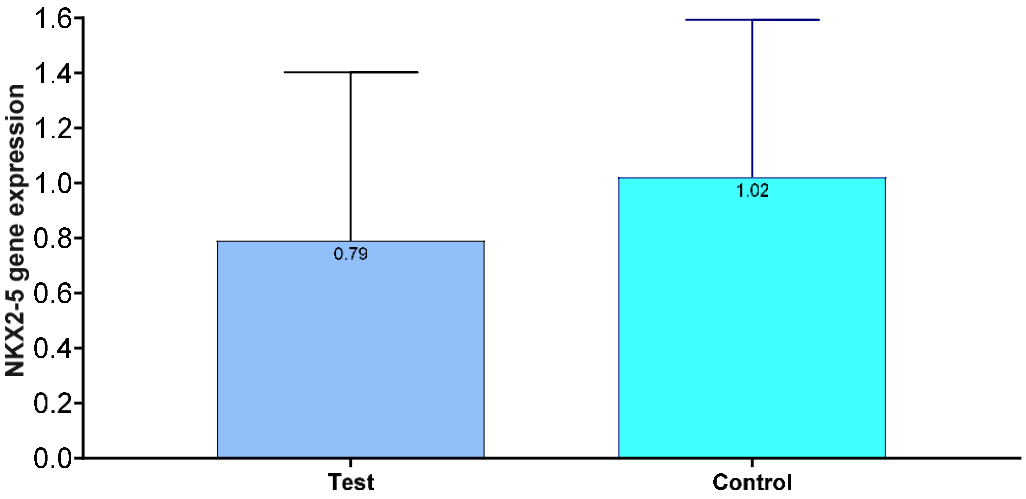
*Values are presented as means ± standard deviations. Statistical significance was determined using independent sample t-tests with p-values < 0.05 considered significant.

Table 1: Association of Demographic, Physiological, Biochemical, and Genetic Parameters

In the genetic analysis, Table 1 revealed the NKX2-5 gene expression levels in both the case and control groups. An intriguing finding was the significantly lower expression of the NKX2-5 gene in the heart failure group (0.79 ± 0.612 v/s 1.021 ± 0.573). This suggests a potential genetic component in heart failure susceptibility. The P value of 0.015 suggests that there is little possibility that this change happened by chance, indicating that the observed variation in NKX2-5 gene expression is probably significant and might have something to do with this condition or treatment under investigation. The NKX2-5 gene is recognised to perform a crucial function in cardiac development and function, and its reduced expression could contribute to increased heart failure risk. The mean level of NTproBNP in the test group was, 135.8±41.3 compared to 73.2±32.2 in the control group (p < 0.001). In previous history, NTproBNP level over 900pg/ml may be sign of heart failure. These findings align with certainly contributing to diagnostic methods. Furthermore, IL-6 were significantly higher levels of IL-6 (6.83 ± 3.2 v/s 1.97 ± 1.58), the mean level in the case group was exceeds the normal value, so these findings support the involvement of inflammation and cardiac stress in heart failure. Elevated IL-6 levels are often associated with chronic inflammation, infections, and various inflammatory conditions.



NKX2-5 gene expression



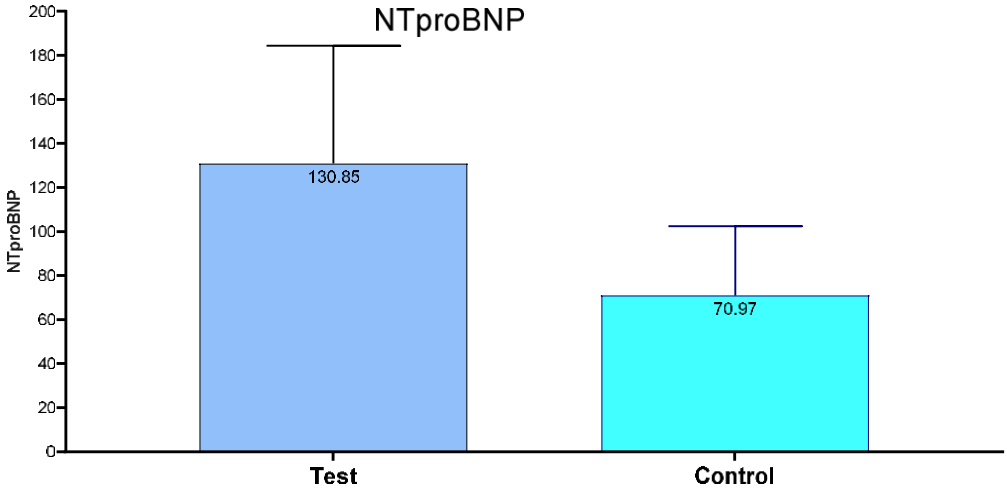


Figure 1, 2 & 3: Comparison of IL-6, NTproBNP, and NKX2-5 Levels between Test and Control Groups
The bar diagram shows (Figure no- 1, 2, & 3) that the test group has notably greater IL-6 levels in comparison to the Control group, , with bars reaching approximately 7 and 2, respectively. The NKX2-5 gene expression level are lower in test group ranging from 1.5 to 2. Similarly, the box plot of NTproBNP suggest that test group has higher levels compared to control, the bar reaching approximately 130 to 70 in separately. These graphs suggest that the test group has increased IL-6 and NTproBNP due to the applied conditions.

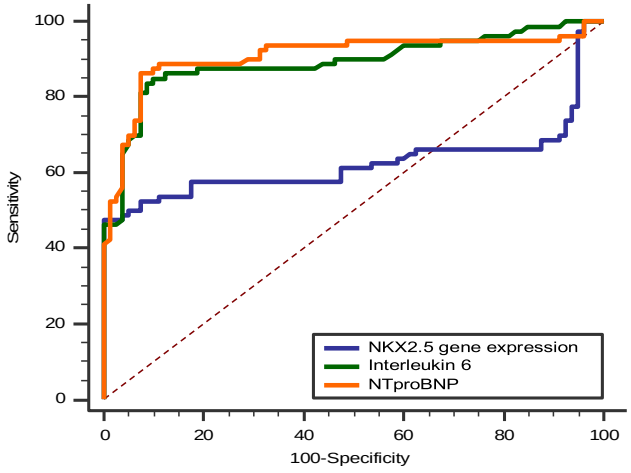


Fig-4- ROC analysis of predictors of cardiac failure

The ROC curve analysis, depicted in Figure 4, demonstrates how different biomarkers perform in terms of diagnostic performance by graphing the true positive rate (sensitivity) versus the false positive rate (specificity). The region beneath the curve, or AUC. The AUC (Area Under the Curve) values reflect the discriminative power of each biomarker. The highest AUC value, signifying its superior ability to differentiate between the test and control groups. With a high Area Under the Curve (AUC) and great diagnostic accuracy, NTproBNP is a useful marker for the condition under investigation. NKX2-5 is less effective than NT-proBNP and IL-6, yet it still has some diagnostic effectiveness due to its moderate AUC. With an adequate AUC, IL6 is another reliable biomarker for the disease under investigation.

	AUC	SE	95% CI
NKX2.5 gene expression	0.625	0.0498	0.545 to 0.700
Interleukin-6	0.892	0.0275	0.833 to 0.935
NTproBNP	0.909	0.0265	0.853 to 0.948

Table-2: **Area Under the Curve (AUC)** values for different biomarkers

The ROC curve analysis provides a comprehensive evaluation of the diagnostic biomarkers. The Area Under the Curve (AUC) values indicate that NTproBNP is the most effective biomarker, with an AUC of 0.909, suggesting diagnostic marker. This is further supported by its low standard error (SE) of 0.0265 and a narrow 95% confidence interval (CI) ranging from 0.853 to 0.948. NKx2-5 gene reflected by an AUC of 0.625 and SE of 0.0498, and 95% CI of 0.545 to 0.700 showing reasonable precision. IL6 with AUC values 0.892 and SE 0.0275, IL6 has 95% CI of 0.833 to 0.935. Moreover, the curves for NTproBNP and IL6 suggest they are both strong predictors, while NKX2-5 gene expression is a moderately effective predictor.

All three parameters demonstrate strong diagnostic capabilities for heart failure, with NTproBNP showing the highest AUC, followed closely by IL6 and NKX2.5. The confidence intervals further support the robustness of these findings, as they do not include the p value of 0.5, which would indicate no diagnostic ability. Thus, these markers can be considered valuable tools in the clinical assessment of heart failure risk.

	B	S.E.	p	**Odds ratio (OR)**	95% C.I.for OR	
					Lower	Upper
H/o Diabetes	3.288	0.962	0.001	26.8	4.1	176.6
H/o Hypertension	-1.169	0.918	0.203	0.3	0.1	1.9
H/o Dyslipidemia	2.119	0.866	0.014	8.3	1.5	45.5
H/o Ischemic heart disease	0.613	0.855	0.473	1.8	0.3	9.9
H/o Thyroid Disorder	0.98	0.749	0.191	2.7	0.6	11.6
Interleukin-6 (IL-6)	-0.719	0.184	0.000	0.5	0.3	0.7
NT-proBNP	-0.034	0.012	0.005	1.0	0.9	1.0
Constant	-3.523	2.45	0.151	0.0		

Table 3: Binary Logistic Regression Analysis of Parameters between Test and Control Groups

*Statistical significance is indicated by p-values (<0.05), with adjusted odds ratios (Adj.OR) and 95% confidence intervals (CI) provided for multivariate analysis.

The binary logistic regression analysis was conducted (table no-5) to evaluate the tandem between various factors and the likelihood of heart failure, based on the results from the univariate analysis that identified significantly associated factors. The model included several variables, each contributing to the prediction of heart failure risk. A higher probability of the result is substantially associated with a history of diabetes (OR = 26.8). Diabetes patients have odds that are 26.8 times higher than those without the disease, and there is a 95% confidence level. (Adj. OR:26.8, 95%CI 4.1 -176.6, p<0.001)

The outcome is not significantly correlated with a history of hypertension (Adj. OR: 0.3 95% CI OF 0.1-1.9 p > 0.05). Although individuals with hypertension appear to have lower odds of the outcome, the OR of 0.3 indicates that this finding is not statistically significant. Significantly greater odds of the outcome (Adj.OR: 8.3 95%CI 1.5-45.5 p > 0.05) are linked to a history of individuals with dyslipidaemia having risks that are 8.3 times higher. The outcome does not significantly correlate with a prior history of thyroid issues(p > 0.05). Increased odds are shown by an OR of 2.7, yet this outcome is not statistically significant. There appears to be

a protective effect as higher levels of IL-6 have been associated to lower chances of the outcome (Adj.OR:0.595%CI of 0.3-0.7 p<0.001). There is statistical significance in the outcome and NT-proBNP has an insignificant affect size (Adj.OR:1, 95%CI of 0.9-1.0 p<0.001). Interestingly, the table showed an unexpected inverse relationship between IL-6 levels and heart failure risk, which warrants further investigation.

Correlation of IL 6 with other parameters	Pearson correlation coefficient	
	r	p
NKX2-5 gene expression	-0.040	0.726
NTproBNP pg/mL	.342	0.002
Fasting Blood Sugar mg/dL	.462	<0.001
Serum Triglyceride mg/dL	.325	0.003
Serum Total Cholesterol mg/dL	.348	0.002
Serum HDL Cholesterol mg/dL	-0.152	0.178
Serum LDL Cholesterol mg/dL	.370	0.001
T3 pmol/L	-.422	<0.001
T4 nmol/L	-.416	<0.001
TSH µIU/mL	.467	<0.001

Table-4: Correlation of IL 6 with other parameters

Correlation of NTproBNP with other parameters	Pearson correlation coefficient	
	r	p
NKX2-5 gene expression	-0.171	0.130
IL 6 pg/mL	.342	0.002
Fasting Blood Sugar mg/dL	.220	0.050
Serum Triglyceride mg/dL	0.104	0.361
Serum Total Cholesterol mg/dL	0.163	0.149
Serum HDL Cholesterol mg/dL	-0.095	0.401
Serum LDL Cholesterol mg/dL	0.188	0.094
T3 pmol/L	-.310	0.005
T4 nmol/L	-.340	0.002
TSH µIUm/L	0.140	0.215

Table-5: Correlation of NTproBNP with other parameters

Correlation of NKX2-5 gene expression with other parameters	Pearson correlation coefficient	
	r	p
NTproBNP pg/mL	-0.171	0.130
IL 6 pg/mL	-0.040	0.726
Fasting Blood Sugar mg/dL	-0.068	0.547

Serum Triglyceride mg/dL	-0.044	0.698
Serum Total Cholesterol mg/dL	0.164	0.146
Serum HDL Cholesterol mg/dL	0.171	0.129
Serum LDL Cholesterol mg/dL	0.173	0.124
T3 pmol/L	-0.023	0.839
T4 nmol/L	0.032	0.777
TSH µIU/mL	0.063	0.580

Table-6: Correlation of NKX25 gene expression with other parameters

The tables (table no- 4, 5 &6) illustrating the relationships between IL-6, NTproBNP, and NKX2-5 with various physiological and metabolic parameters (Table 3, 5 and 6). IL-6 showed positive correlations with several metabolic markers, fasting blood sugar, triglycerides, total cholesterol (r=.462, r=.325, r=.348, p <0.001) and a negative correlation with thyroid hormones (T3, T4) (r= -422, -416, p <0.001). Although there was no statistically significant correlation was found in NKX2-5, NTproBNP, serum HDL cholesterol, This is evident from their non-significant p-values (p > 0.05). On other hand NKX2-5 has a significant negative correlation with NTproBNP, IL-6, Fasting blood sugar, Serum Triglycerides, and T3. These results point to complex interactions. between inflammation, metabolism, and endocrine function in the context of heart failure.

DISCUSSION

Heart failure is a complex illness that develops when the heart is unable to adequately pump blood to meet the body's metabolic demands. .The findings of this study revealed a significant association between gene expression levels and biomarkers IL6, NTproBNP levels and cardiovascular disease outcomes. IL-6 is generally accepted as a marker of inflammation and high levels of IL-6 contributes to cardiovascular disease, the result of this study suggest a more complex interplay that requires careful consideration. According to the study, elevated IL-6 levels in heart failure patients are a significant pro-inflammatory indication for the onset of cardiovascular disease (18). A study suggest that elevated IL-6 might be considered as a significant independent predictor of HF, and it could be also be used as a predictive biomarker in HF (19). Additionally, IL-6 is considered an independent marker for poor outcomes in heart failure patients (20). This current study supports these findings, showing a strong relationship between IL-6 and heart failure.

The study findings of Ghamri RA et al. support the assumption that IL-6 and CRP level are involved in MI but their correlation with cardiovascular biochemical risk factors, was not found (21). On the contrary, the present study showed positive relation of IL-6 with several markers like fasting blood sugar (p-<0.001), trigycerides (p-0.003), total cholesterol (p-0.003), and a negative correlation with Thyroid hormone T3 (p-< 0.001) and T4 (p-<0.001). These results point to complex interactions between inflammation metabolism, and endocrine function in the context of heart failure.

NT-proBNP levels are useful tool diagnostic tool for heart failure and possibly even determine the prognosis of the condition and NT-proBNP acts a biomarker in HF management and highlights the therapeutic benefits of sacubitril/valsartan in reducing NT-proBNP levels and improving patient outcomes (22). The findings of a study implies that detecting NT-proBNP in ambulatory people without baseline cardiovascular disease (CVD) can give useful predictive information. Increased amounts of NT-proBNP in patients with modestly raised blood pressure (120 to 149 mm Hg) may suggest an increased risk for certain cardiovascular (CV) events (23). Diastolic dysfunction is significantly predicted by age and NT-proBNP levels (24). NT-proBNP has a better prognosis, particularly in younger and female participants (25). An earlier study shows the potential efficacy of enhanced treatment depending on NT-proBNP levels for individuals at high risk of cardiac decompensation (26). NT-proBNP is suggested as a biomarker for prognosis throughout the whole course of the disease and

diagnosis in both acute and chronic symptomatic heart failure (HF)(27). Current study finding shows that NTproBNP emerged as a significant predictor of heart failure and it is consistent with findings from other studies, which have reinforced the prognostic significance of NTproBNP in heart failure outcomes.

Elevated IL-6 levels in patients with acute heart failure correlate with elevated NT-proBNP levels, providing predictive information for overall mortality (28). Studies have shown that NT-proBNP levels and IL-6 have a nonlinear relationship., indicating that elevated IL-6 levels are related with increased NT-proBNP levels (29). The present study's correlation analysis also underscores the relationship between IL-6 and NTproBNP (P= 0.002). These findings support the results of previous studies, reinforcing the link between IL-6 and NT-proBNP levels in heart failure.

Cardiac transcription factors are key in activating cardiac gene expression and regulating genes for specific structural or regulatory proteins. NKX2-5, a crucial gene in cardiogenesis, helps in precursor cell differentiation, cardiac development, and plays a vital role in various physiological functions (30). The studies reveals that functional insufficiency or lower levels of NKX2-5 and the role of NKX2-5 gene continues throughout adulthood and stresses how crucial it is to the healthy operation of the adult heart (31).

Several animal model studies was explore the function of NKX2-5 gene expression in adult heart function and maintenance. A study demonstrates that NKX2.5 increases mesenchymal stem cells (MSCs) in rats treated for heart failure. NKX2.5 promotes MSC survival, differentiation into cells resembling cardiomyocytes, strengthens the heart, lessens fibrosis, and upregulates the expression of MEF2 and GATA4 (32). Another study reveals that a reporter gene in the heart is activated by NKX2-5 binding to certain locations in the ANF gene, in heart failure patients' NKX2-5 chromatin hub formation is insufficient to reactivate ANF (33). A study suggests that NKX2-5 may inhibit p53-mediated cell death in hyperglycemia, potentially increasing cardiomyocyte lifespan by controlling p53 activity and lowering cardiac damage (34). Through the activation of cardiac-specific embryonic genes, an increase in eGFP+ cells after myocardial infarction (MI) in the NKX2-5 cardiac enhancer-eGFP transgenic mouse model revealed progenitor characteristics (35). A study finding demonstrated that postnatal cardiomyocytes treated with overexpressing NKX2-5 and its mutants underwent structural and functional changes, with connexin 43 levels rapidly rising in response to increased wild-type NKX2.59(36). As a regulator of several ion channel gene expression, NKX2-5 is essential for perinatal conduction and contraction(37). The findings of these studies suggests that NKX2-5 is essential to the upkeep of adult cardiac function and is implicated in various cardiac diseases, for example NKX2-5 insufficiency has been linked to atrial electrical remodeling, increasing the risk of arrhythmias and heart failure (38). In the current study, the case group's NKX2-5 gene expression was lower than that of the control group's, suggesting that NKX2-5 downregulation may elevate the risk of heart failure.These results align with earlier research , supporting the role of NKX2-5 expression in the pathogenesis of heart failure. However, confirmation in a larger sample size is necessary.

The present investigation highlights the potential of NKX2-5 gene expression in the diagnosis of heart failure, given its notable diagnostic accuracy in comparison to well-established indicators as NT-proBNP and Interleukin-6 (IL-6). NKX2-5 performs significantly in the diagnostic landscape while having an AUC of 0.625, which indicates modest discriminative capacity. On the other hand, NT-proBNP and IL-6 have larger AUCs of 0.909 and 0.892, respectively. The near performance of NKX2-5 highlights its significance in clinical applications, even if IL-6 shows a somewhat superior AUC. This is especially true considering that NT-proBNP's AUC, however higher, is only marginally better than NKX2-5. This emphasises how useful NKX2-5 may be as a biomarker for heart failure diagnosis.

Conclusion

Incorporating NKX2-5 gene expression testing in standard clinical evaluation can improve early heart failure diagnosis and personalized treatment. Its strong diagnostic performance complements existing biomarkers IL6 and NTproBNP, offering a promising avenue for advancing heart failure diagnosis. Genetic counselling could

offer individualised insights into the treatment and early identification of heart failure, as well as help patients understand their risk based on genetic markers such as NKX2-5. Further research is needed to explore NKX2-5's impact in comprehensive diagnostic panels and patient outcomes.

However, several limitations exist in the current investigation. A limited sample size and the fact that the sample was drawn from a single centre restrict how broadly the results may be applied. The growth of heart failure and the impact of treatment on marker level are not examined by the study's cross-sectional approach. Finally, additional research in larger more diverse populations is necessary to validate NKX2-5's potential as a diagnostic marker.

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