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Study of Total RNA Circulation and Tryptase Activity Levels in Heart Failure Patients

Nagham Qasim Kadhim[1], Fawzi Hassan Zayr[2], Sabah Fadhil Al-Qurashy[3]

1 Department of Biochemistry, College of Science, Tikrit University, IRAQ
2 Department of Clinical Biochemistry, College of Medicine, Wasit University, IRAQ.
3 Department of Medicine / College of Medicine / Wasit University, IRAQ.

ABSTRACT

***Background**: Heart Failure (HF) take place when heart muscle couldn't pump blood as it should. HF is a clinical disorder that occurs due to structural and functional weakness in the myocardium leading to a weak ventricle in the blood pump.* ***Methods**: Serum Tryptase and Total RNA Blood were taken from 90 samples: 45 sample with heart failure, and 45 normal healthy control. The age of individual ranged from (40-65) years. The tryptase assay by ELISA technique. Total RNA Blood was determined by QIAamp RNA Blood Mini Kits.* ***Results**: There are a very high significant increase in activity of tryptase and a high significant decrease in the total RNA concentration in whole blood in heart failure patients group, in addition to a very high significant decrease in the total RNA levels in whole blood heart failure patients group when compared with normal subject. A highly significant in the activity of tryptase and a highly significant in the total RNA levels in whole blood in heart failure (male, female) patients group when compared with (male, female) normal subject. There are a highly significant in the serum activity of tryptase and highly significant in the total RNA concentration in whole blood in patient group according to compared it with normal subject in age 38-50 years and in age >50.*

***Conclusions:** There was a correlation between tryptase levels and circulation total RNA levels and development of major adverse HF events.*

KEYWORDS; Heart Failure, Tryptase, Total RNA

INTRODUCTION

Heart failure (HF) is a chronic condition (syndrome) produced in which happen when exist a structural and functional disturbances in myocardium leading to retrogradation and weakness of ventricular padding. The greatest public cause is reducing function of left ventricular; yet, abnormality or impairment in the function of a pericardium, great vessels alone or with HF, myocardium, endocardium or heart valves.

Some of the main mechanisms which produce HF are enhanced blood flow overload, ventricular rebuild, undue neurological and humoral stimulation, anomalous myocyte calcium recycling, insufficient reproduction of extracellular matrix, faster apoptosis, ischemia dysfunction, and genetic mutations (1).

Tryptases are enzymes that belong to the serine proteases group, tetrameric, excrete by human mast cells , possess a molecular weight of approximately 134 kDa (monomer approximately between 26-35kDa)(2),(3). The amount of tryptase (EC 3.4.21.59) from total protein content of mast cell granules approximately 25%, the tryptase are stored in active shape before release through mast cell degranulation. The tryptase working show be constrained to the extracellular milieu (4). The arranging of tryptase active side subunits results a little oval central pore (It's size approximately 50×30 $^{\circ}$A), which lead to restrictive accessibility for substrate and inhibitors (5). The tryptase monomer is coordinated with six externally uncovered, that associated with its external circumference, inclusive its adjacent monomers, when in the tetramer form. These domains are external loops. Named are: "37-loop", "60-loop", "70- to 80", "97", "147", and "173" loop. Because of this loops encircle the active site of tryptase, any alteration in these domains can make hard modify in the specificity of this enzyme. Tryptase, also, includes a catalytic umvirate, that is fundament for its proteolytic activity (6), (7). Multiple human tryptases have been specified, inclusive α, β, δ, γ and ε (8), yet, suspicion occur to regardless all structures or forms are efficient and functional. The genes that encoded human tryptases are a cluster or Located on a 2.5Mb region, on the small arm (p) of chromosome 16, (16 p13.38).

A noncoding RNA (ncRNA) is an RNA molecule which is transcribed from DNA, but don't use to build proteins. Generally, ncRNAs function to organizing gene expression at the all levels. These ncRNAs which appear to be take part in epigenetic practicability can be split into 2 major groups; ncRNAs (<30 nts -the short) and ncRNAs (>200 nts -the long) (9),(10). Latterly, attention in ncRNAs has start increasing because they represent the performance majority of the transcriptase (11), (12) and also because of their relationship with cardiovascular diseases (13-15). The ncRNAs are set up to stable and determined in fluids of body, they appear a possibilities and benefits for used as biomarker parameter for diagnostic scientific applications. Diverse new research have found that: extracellular rRNA and miRNAs, significantly participate to the opposite output of cardiovascular impairment. Extracellular RNAs working as new risk -sensor linked molecular signals and powerful cofactors in thrombosis and inflammation in cardiovascular system, particular when gather in the extracellular area around tissue- damaged or in pathological cases (16),(17).

MATERIALS AND METHODS

Forty five patients (23 males and 22 females) suffering from heart diseases were participated in the present study. Their ages ranged from 40 to 65 years. Samples were collected from Wasit General Hospital and the Salah al-din Hospital (Al-Askari) in Tikrit district, during the period from April to August 2017. All patients were diagnosed by a specialist doctor, and each patient have troponin T was positive.

The control group was 45 apparently healthy controls (25males and 20 females). Ages of the involved subjects ranged from (40-65) years. Five milliliters (5ml) of blood were drown from each patient and healthy control individuals. Serum was obtained and kept into small Eppendorf tubes capacity 1.5 ml at -20C° until time of analysis. The tryptase assay employs by the ELIZA technique (18). Total RNA Blood was determined by QIAamp® RNA Blood Mini Kits (19) (QIAGEN company) which designed for extraction of total RNA from novel, human whole blood which collected in anticoagulant(EDTA tube) for estimation of RNA concentration.

Statistical analysis: The probability (P values= significance of difference) were estimated by student T-test.

RESULTS AND DISCUSSION

The mean (±SD) of tryptase activity and total RNA in normal healthy control group and patients with heart failure group are explained in table [1]. There are a highly significant difference in the activity of tryptase in heart failure patients group according to compared with normal healthy group (control), and a highly significant decrease in the total RNA concentration in whole blood heart failure patients group compared with normal healthy group (control).

Table [1]: Mean (±SD) tryptase activity and total RNA concentration in study group

Parameters	Control	Heart failure	p
Tryptase (ng/ml)	16.6±8.19	49.88±6.24*	0.0001
Total RNA (ng/ml)	212±31.23	88±12.90*	0.0001

Table [2] : Shows there are a highly significant rise in the activity of tryptase in heart failure male patients when compared with (male) control, and a highly significant rise in the activity of tryptase in heart failure female patients when compared with (female) normal control, while , no significant different between male and female

Also, there are a significant decrease in the concentration of total RNA level in whole blood heart failure male patients group as compared with (male) control group, and a significant decrease in the total RNA level in whole blood heart failure female patients group as compared with (female) control group. There are a significant decrease in the total RNA level in whole blood heart failure female patients group when compared with male patients group.

Table [2]: Mean (±SD) tryptase activity and total RNA level in study group according to gender.

Parameters		Control	Heart failure
Tryptase (ng/ml)	male	17.68±2.33	44.18±9.23*
	female	16.06±2.12	46.88±12.34*
Total RNA (ng/ml)	male	229±21.03	101±5.50*
	female	195.01±10.20	75.99±6.63*#

*High Significant p<0.0001 # Significant p<0.05 compared with males

Table [3] shows there are a significant increase in the activity of tryptase in patient group compared with normal healthy control group in age 38-50 years, there are a significant increase in the activity of tryptase in patient group compared them with control group in age >50 , and no significant different between 38-50 years and >50 age groups.

Also, there was a very high significant decrease in total RNA level in whole blood patient group when compared it to control group in age 38-50 years, and significant decrease in the total RNA level in whole blood patient group as compared to control group in age >50, and significant decrease in the total RNA level in whole blood patients group in age>50 when compared with 38-50 years patients group.

Table [3] : Mean (±SD tryptase activity and total RNA concentration in study group according to age.

Parameters		Control	Heart failure
Tryptase (ng/ml)	38-50 years	17.68±2.33	44.18±9.23*
	>50	16.06±2.12	46.88±12.34*
Total RNA (ng/ml)	38-50 years	242±21.23	100±12.11*
	>50	181.05±24.43	76.99±18.63#

*High Significant # Significant

Mast cells consist of various excess mediators that gives them the capacity to do diverse mechanisms. For the mast cells to work, effecting and to stimulate plaque progression (41), they require to be activated to liberation and release mediators. Interesting: one of the main plaque damaging effects is by the mast cell–tryptases (9). In different studies, tryptase levels in plasma have been shown relate to cardiovascular diseases. However, another searches failed show any change in tryptase levels pending cardiovascular events (20). As yet, serum tryptase is increasing extent used as a signature for different abnormalities in clinical exercise (4). Despite of studies detects a positive relationship between obesity and raise mast cells number for both animals and humans (41). Prior doings show a lineal contribution for mast cells in insulin resistance and type 2 diabetes mellitus (T2DM) in animal models (21).Numerous experimental studies have found that: tryptase has a important role in the atherosclerosis in addition in the formation of aortic aneurysm (42). Zhang et al. found a decrease in formations abdominal aortic aneurysm appear in tryptase imperfect animals (22). Newly

demonstrated that, inhibition tryptase by medications decrease abdominal aortic aneurysm (23). The relationship between increase tryptase concentration and the development events cardiovascular could lead to prove that: the mast cells have important role in the weakness and degradation process to heart. Others explained that: powerful roles in activated and functioning mast cells in the growing destabilization of a plaque (24).

In this time, many researchers have given their concern to the RNA domain. With the common use of RNA-sequencing, the finding is ncRNAs related to disease expansion. Circulating ncRNAs are powerful serve as index for disease valuation. Invert proteins, RNAs are more stable in the organ and also in blood (25). Only 2% of genomes are responsibility of coding for protein. RNAs are grouped as ncRNAs (26). Although this, ncRNAs are very important because they participate in protein silencing, modification and promotion (27). Emerging evidence has confirmed a close correlation with pathophysiology for human heart (28). Consecutive studies found different specific lncRNAs that are high sensitive which attached to disease progress (29).

The existence of DNA (RNA) outside the cell and sour the DNA in blood is usually present as a result of turnover of cells or by the formation proses of micro vesicles blood cells (30), platelets, and secretive cells remain in tissues such as placenta, organs ,endocrine, tumors, tissue injury especially in liver (32),(32). Considering the change in concentration of free nucleic acids, and extracellular their composition reflect to their unequalled metabolism and disease operation (33), and identical structural sequence has been considered beneficial for the determination of novel biomarkers (34-35). That damage to the heart tissue will result in excess release of ncRNA similar to secretion of protein. For improvement and complementation such as the associated biomarker, miRNA transiently and recently inRNA and potential circular IncRNA are expected to reflect equivalently heart damage, the Engage of other organ, and generally patient disease (36, 43). Different reports supply evidence that RNAs is involved in the growing and forward of HF, the study of circular RNA has been studied as a biologically increasing of possible heart failure has been summarized by various references (37-39).

Conclusion: Studies are ensured to prove that blood RNA can be used as biomarkers for cardiac damage, especially HF, we agree with other (40) which found that “blood cells act as sentinels of some disease.” Therefore capitalize from it for the diagnosis, or follow them with cardiac diseases.

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ABBREVIATION USED

Heart Failure (HF), kilo Dalton (kDa), histamine(His), Asparagine(Asp), Serine(Ser) .

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