

## Role of Tryptase in Cardiorenal Syndrome

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### ABSTRACT

**Background:** Acute heart disease patients often go on to develop worsening renal function, termed as cardiorenal syndrome. The growing breadth of studies has shown the implications of combining multiple biomarkers to better chart outcomes between heart and kidneys, and produce desirable results in such patients, as tryptase. **Aim of the study:** To estimate the levels of tryptase, and their relationship with cardiorenal syndrome. **Methods:** Tryptase were obtained from 62 samples: 40 sample with cardiorenal syndrome, and 22 normal healthy. **Results:** A highly significant increase ( $p < 0.0001$ ) in the levels of tryptase in serum of patients with cardiorenal syndrome, heart disease and kidney disease compared with normal individuals. **Conclusions:** Tryptase has higher diagnostic validity values in the current study, which may be useful as a diagnostic tool to identify recurrence of the cardiorenal syndromes.

**KEYWORDS:** Cardiorenal biomarkers, Acute heart disease, Tryptase.

### INTRODUCTION

The tight and delicate coordination of physiological functions among organ systems in the human body is such that a dysfunction in one could lead to malfunction of one or more organ systems, this relationship know as cardio-renal syndrome (CRS)<sup>1</sup>. Both heart and the kidneys are richly vascular (the kidneys are more vascular than the heart) and both organs are supplied by sympathetic and parasympathetic innervations. These two organs act in tandem to regulate blood pressure, vascular tone, diuresis, natriuresis, intravascular volume homeostasis, peripheral tissue perfusion and oxygenation<sup>2</sup>. They have endocrine functions with interdependent physiological hormonal actions regulated by arterial natriuretic peptide, a vasodilator secreted from the heart and renin-angiotensin-aldosterone system. Also, vitamin D3, erythropoietin etc. are all secreted from the kidneys, and are capable of cellular and humoral signaling. Dysfunction of either of the two organs can cause dysfunction of the other<sup>3</sup>.

Mast cells are granulated effector cells of the immune system and are believed to play a role in inflammation, tumor angiogenesis, immunoregulation, and tissue repair. Tryptases are active at neutral pH, and their production helps distinguish mast cells from other leucocytes<sup>4,5</sup>.

Tryptases (EC 3.4.21.59) are tetrameric serine proteases enzymes secreted by human mast cells and have a

molecular weight of ~ 134kDa (monomer ~ 26-35kDa)<sup>5,6</sup>. Tryptases account for ~ 25% of the total protein content of mast cell granules, and are stored in their active form prior to release during mast cell degranulation. However, their actions appear to be restricted to the extracellular milieu<sup>7</sup>.

The arrangement of tryptase's catalytically active subunits produces a small oval central pore (size~50×30Å), resulting in restricted accessibility for substrates and inhibitors<sup>8</sup>. The tryptase monomer is arranged with six externally exposed domains, which interact with its external environment, including its neighboring monomers, when in the tetramer formation. These domains are tryptase's surface loops, and are named the "37-loop", the "60-loop", the "70- to 80-loop", the "97-loop", the "147-loop", and the "173-loop". As these loops surround the active site, any changes in these loops can potentially alter the substrate specificity of tryptase. In addition, tryptase contains a catalytic triad (His-57, Asp-102, Ser-195 (chymotrypsin numbering1)), which is essential for its proteolytic activity<sup>9,10</sup>.

Multiple human tryptases have been identified, including  $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\gamma$  and  $\epsilon$ <sup>8,11</sup>, however, uncertainty exists as to whether all forms are functional. The genes that encode human tryptases are located in a cluster within a 2.5Mb region, on the short arm of chromosome 16, at position

16 p13.3<sup>8</sup>. All known human tryptase genes have a six exon/five intron organization, which is approximately 1.8kb long. This gene architecture differs from that of other mast cell or leucocyte serine proteases. Tryptases contain a 30-amino acid prepropeptide followed by a 245-amino acid catalytic domain. Although the 5L regulatory region is similar to other serine proteases, it is unique due to its separation from the initiator Met codon by the first intron. Tryptases have been found in several mammals (e.g. human, dog, mouse, rat, gerbil, sheep and cow)<sup>9,2</sup>. Although closely related in primary structure,  $\alpha$  and  $\beta$ -tryptase differ markedly in their biochemical properties.  $\beta$ -Tryptase isolated from mast cells or produced in recombinant form is a tetramer composed of four identical catalytic subunits<sup>12</sup>.

Although a number of substrates have been identified for tryptases in vitro, their true biological roles and targets are still unclear. However, they are reported to induce microvascular leakage and inflammatory cell accumulation, and regulate mast cell activation. They are therefore important mediators of inflammation<sup>8</sup>, and have a prominent role in diseases such as asthma, inflammatory bowel disease, and rheumatoid arthritis<sup>13</sup>.

In addition, tryptase activity can be inhibited by synthetic protease inhibitors, and several such therapeutic approaches have shown clinical efficacy in the treatment of asthma and ulcerative colitis<sup>14</sup>. It is reported that tryptase may contribute to vascular permeability by the direct or indirect generation of bradykinin from kininogens. Mast cell tryptase increases intracellular  $Ca^{2+}$ , leading to elevation of paracellular permeability of colonocytes. Intradermal injection of tryptase or mast cell secretagogue compound 48/80 in rats can induce the immediate cutaneous reaction and increase dermal microvascular permeability, which can be inhibited by potent and specific tryptase inhibitor nafamostat or synthetic tryptase inhibitor<sup>15</sup>.

## MATERIALS AND METHODS

Thirty-eight patients (20 males and 18 females) suffering from cardiorenal syndrome, heart diseases, and kidney diseases were participated in the present study. Their ages ranged from 38 to 70 years. Samples were collected from Wasit General Hospital and the Medical City Hospital in Baghdad during the period from August to November 2014. CRS as a condition in which therapy to relieve congestive symptoms of heart failure is limited by a decline in renal function as a result from a reduction in renal blood flow. All patients had blood urea level  $>60\text{mg/dl}$  and serum creatinine  $>2\text{mg/dl}$ . Ethical approval was obtained from the patients or their first degree relatives according to Iraqi IRP. The control group was 22 apparently healthy controls (10 males and 12 females). Ages of the involved subjects ranged from (38-70) years. Five milliliters of venous blood were drawn from each patient and healthy control individuals.

Serum was obtained and kept into small Eppendorf tubes capacity 1.5 ml at  $-20\text{C}^{\circ}$  until time of analysis. The tryptase assay employs the quantitative sandwich enzyme immunoassay technique<sup>16</sup>.

**Statistical analysis:** The significance of difference between mean values were estimated by student T-test. The probability is considered as significant when  $p<0.05$ . The data were processed with the software package XLSTATE (2015) and Microsoft Excel 2010 to estimate the receiver operating characteristics (ROC) curves and cut off values.

## RESULTS AND DISCUSSION

The mean ( $\pm\text{SD}$ ) of tryptase concentration in serum of control group (healthy individuals) and serum of patients with cardiorenal syndrome group are shown in table-1. There are a highly significant increase ( $p<0.0001$ ) in the serum levels of tryptase in cardiorenal syndrome group when compared with control group, and a significant increase ( $p<0.05$ ) in the serum levels of tryptase in males when compared with females, while there was no significant differences between age groups as shown in the table-2.

**Table 1: Tryptase concentration in study group according to gender.**

Tryptase	Control	CRS
<b>Total</b>	<b>16.6<math>\pm</math>3.19</b>	<b>49.88<math>\pm</math>6.24*</b>
<b>Males</b>	<b>15.4<math>\pm</math>2.12</b>	<b>52.89<math>\pm</math>8.38<sup>#</sup></b>
<b>Females</b>	<b>14.99<math>\pm</math>1.15</b>	<b>43.87<math>\pm</math>9.11</b>

\*high Significant  $p<0.0001$  compared with control  
<sup>#</sup>significant  $p<0.05$  compared with females

**Table 2: Tryptase concentration in study group according to age.**

Tryptase	Control	CRS
<b>38-50 years</b>	<b>15.6<math>\pm</math>2.10</b>	<b>49.12<math>\pm</math>5.66</b>
<b>&gt;50 years</b>	<b>16.8<math>\pm</math>5.22</b>	<b>47.78<math>\pm</math>9.43</b>

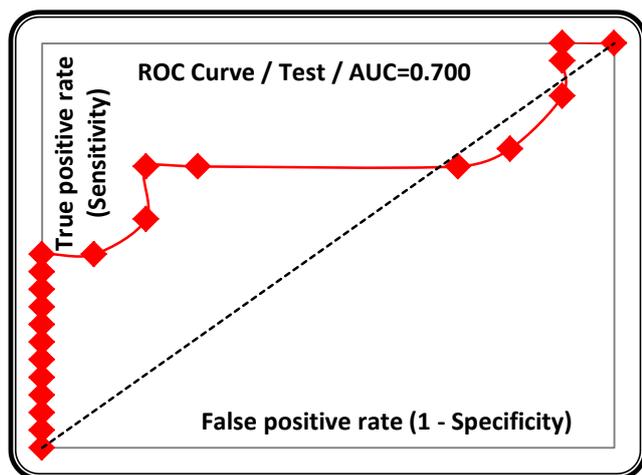
Receiver operating characteristics (ROC) curves were used to compare the performance of the biochemical diagnostic methods of diseases in this study and to determine the appropriate cut off values for the different markers. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to analyze the diagnostic value of each hormone. The area under the curve (AUC) was commonly used as a summary measure of diagnostic accuracy. Table-3 shows the criteria of statistical validity of tryptase level in cardiorenal syndrome group compared with control by using 23.0 ng/ml as cut-off value (The optimal cut-off value for tryptase in all groups estimated from ROC curves). According to these

results, test is positive if test > threshold value (cut off values). Figure-1 explained the ROC curve and for tryptase concentration cardiorenal syndrome group, sensitivity and specificity shown in figure-2. These results indicate that, the copeptin it very sensitive for cardiorenal syndrome.

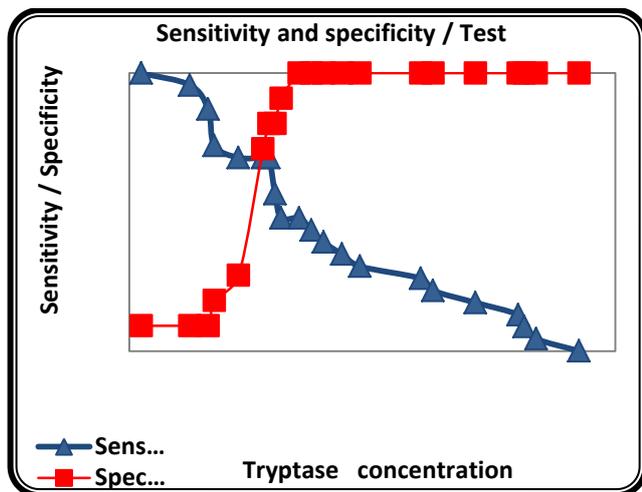
**Table 3: Predictive values of serum copeptin level in G1**

Sensitivity	Specificity	PPV	NPV	Accuracy	AUC*
69%	51%	88%	56%	73%	1.000

\*Area under curve, Copeptin level cut-off value- 23.0 ng/ml.



**Figure 1: ROC curve for tryptase concentration in cardiorenal syndrome group**



**Figure 2: Sensitivity / specificity test for tryptase concentration**

The results demonstrated a significant relationship between the cardiovascular complexity, renal diseases and high tryptase levels. Tryptase is a well-known protease with an established role in immune process. Various mast cell derived mediators are postulated to contribute to plaque destabilization, amongst which are the specific neutral proteases tryptase. In humans, mast cells are divided into two subtypes according to their

protease content, namely MCT cells (mast cells expressing tryptase  $\alpha$  and  $\beta$ ) or MCTC cells (mast cells expressing  $\alpha$  and  $\beta$  tryptase, chymase and carboxypeptidase A), both of which have been identified in atherosclerotic plaques<sup>16,17</sup>. Also, higher plasma tryptase levels were observed in patients experiencing a secondary event, indicative of a predictive value for mast cells in cardiovascular disease<sup>18</sup>. However, conflicting evidence prevails in literature regarding the usefulness of tryptase as a biomarker for adverse events. For example, elevated tryptase levels have been observed in patients with significant coronary artery disease, while a separate other study failed to show differences in serum tryptase levels from patients with acute coronary syndromes as compared to controls. In addition to these studies, several other groups have reported either affirmative or negative findings for the predictive value of tryptase, often limited by a relatively small cohort size. For that, future studies are necessary to appoint a definite role for tryptase as a biomarker in cardiovascular disease<sup>19</sup>.

Secreted tryptases are all capable of activating matrix metalloproteinases, which in turn are responsible for fibrillar collagen degradation. Since one of the roles of the extracellular collagen matrix is to maintain ventricular size and shape, its disruption results in adverse remodeling. Also secreted from the mast cell is a yet to be identified substance that stimulates the maturation of resident immature mast cells<sup>20</sup>.

Bot et al.<sup>21</sup>, recently explained the potential roles of activated mast cells in the growth and destabilization of an atherosclerotic plaque. Mast cells, like other inflammatory cells, are located in the human arterial intima and adventitia, and when activated, they release granules locally, which contain a large panel of mediators, including neutral proteases (tryptase and chymase), cathepsins, heparin, histamine, cytokines and growth factors. During early atherogenesis, the effector molecules stimulate leukocyte recruitment and lipid accumulation in the evolving plaque, whereas during advanced stages of atherogenesis, they contribute to the generation of an unstable plaque susceptible to rupture. Tryptase activates matrix metalloproteinases (MMP), such as MMP-1, MMP-2 and MMP-3 and procollagenase, and promotes the degradation of lipoproteins and fibronectin<sup>21,22</sup>, acting as powerful inflammatory stimulus at the endothelial dysfunction<sup>23</sup>. Many experimental studies have confirmed the role of tryptase in the inflammatory process of atherosclerosis as well as in aortic aneurysm formation. Zhang et al. observed a reduction of abdominal aortic aneurysm formations in tryptasedeficient mice<sup>24</sup>. Wang et al. recently demonstrated that the absence, or pharmacological inhibition, of tryptase reduces abdominal aortic aneurysm formation in animal models<sup>25</sup>. On the basis of these observations, we can suggest that patients with high serum tryptase levels

could have a greater burden of mast cells in the arterial wall. This burden contributes to the coronary lesion formation incidence of acute coronary syndromes and the recurrence of follow-up cardiovascular events. They investigated the relation between serum tryptase levels and markers of inflammation<sup>26</sup>. Mast cells are also present in perivascular tissue where they can regulate vascular functions and activate other inflammatory cells. It is known that high blood pressure may damage the endothelium, increasing its permeability and stimulating the proliferation of smooth muscle cells and of vascular remodeling. Additionally, lymphoid tissue made up of mast cells, among other types, was observed in the intima of carotid arteries at sites of hemodynamic stress. As a matter of fact, tryptase has also been related to the inflammatory activity of atherosclerotic plaques<sup>27</sup>. An interesting finding in chronic kidney disease patients on conservative treatment was the identification of an association between tryptase and certain markers involved in the progression of renal failure, such as uric acid, secondary hyperparathyroidism or proteinuria, as well as other inflammation or endothelial dysfunction mediators in uremia, such as fibrinogen or homocysteine. Although it is a matter of controversy, uric acid seems to be a risk factor of progression rather than a marker of renal dysfunction<sup>28</sup>. Experimental studies suggest that uric acid may stimulate intracellular inflammatory mediators and locally activate the renin-angiotensin-aldosterone system<sup>29</sup>. There are, as well, certain clinical parallels between uric acid and tryptase, based on the relation that they both bear with ischemic heart disease<sup>27,30,31</sup>.

## CONCLUSIONS

It would be interesting to correlate renal mast cell infiltration with plasma tryptase levels to understand the possible pathophysiological pathways involved. The association with renal failure progression markers could underscore the usefulness of tryptase in the overall evaluation of chronic kidney disease patients to classify them and assess their evolution.

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