

## Serum Level of Heart-Type Fatty Acid-Binding Protein in Patients with Chronic Renal Failure

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### قياس معدل بروتين حمض القلب الدهني الرابط في مصاب المرضى الذين يعانون من الفشل الكلوي

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**المخلص: الهدف:** اكتشف جلاتر بروتين حمض القلب الدهني الرابط عام 1988 كأحد العلامات الكيميائية - الحيوية المبكرة الدالة على وجود جلطة قلبية. غير إن تأثير أمراض الكلى على هذا البروتين لم يتم دراسته بشكل واف حتى الآن. هذه الدراسة تقارن تأثير الفشل الكلوي على تركيز بروتين حمض القلب الدهني الرابط و تروبونين القلب (ت) في الدم. **الطريقة:** اشتملت الدراسة على مجموعة مكونة من 16 مريضاً يعانون من فشل كلوي مزمن (ست نساء وعشرة رجال) تتراوح أعمارهم بين 30 - 70 سنة، ويتلقون علاج غسيل كلوي بشكل دوري. **النتائج:** معدل اليوريا والكرياتينين في هذه المجموعة هو  $9.6 \pm 19$  مليمول / لتر و  $531.3 \pm 231.2$  مليمول / لتر على التوالي. كان بروتين حمض القلب الدهني الرابط مرتفعاً عند كل المرضى ( $81 \pm 53.3$  مايكروجرام / لتر، وكذلك كان تروبونين القلب (ت) مرتفعاً بأكثر من 0.1 مايكروجرام / لتر في ثمانية مرضى) 50% ( و أكثر من 0.2 مايكروجرام / لتر في خمسة مرضى) 31% ( و أكثر من 0.3 مايكروجرام / لتر في مريض واحد ) 6%. **الخلاصة:** هذه الدراسة تشير إلى إن قدرة بروتين حمض القلب الدهني الرابط و تروبونين القلب (ت) على تشخيص الجلطة القلبية في وجود فشل كلوي محدود.

**مفتاح الكلمات:** بروتين حمض القلب الدهني الرابط، تروبونين القلب (ت)، فشل كلوي.

**ABSTRACT: Objectives:** Heart-type fatty acid binding-protein (H-FABP) has been reported to be a potential novel biochemical marker for the early diagnosis of acute myocardial infarction (AMI). The effect of kidney diseases on the renal handling of H-FABP has not yet been fully evaluated. The aim of this study was to compare the effect of renal failure on the level of H-FABP and cardiac troponin (cTnT) concentrations. **Methods:** The study population was a small group of 16 patients with renal failure (6 females, 10 males aged 30-70 years) on routine regular haemodialysis or peritoneal dialysis. **Results:** The mean  $\pm$ SD of serum urea and creatinine concentration in this group of patients was  $19 \pm 9.6$  mmol/L and  $531.3 \pm 231.2$  mmol/L respectively. H-FABP was increased in all 16 patients ( $81 \pm 53.3 \mu\text{g/L}$ ). The cTnT was increased  $\geq 0.1 \mu\text{g/L}$  in 8 patients (50%),  $\geq 0.2 \mu\text{g/L}$  in 5 patients (31.3%), and  $\geq 0.3 \mu\text{g/L}$  in 1 patient (6%). **Conclusion:** The diagnostic efficiency of H-FABP and cTnT for the diagnosis of AMI in the presence of renal failure may be limited and such patients may have high levels even in the absence of AMI.

**Keywords:** Heart-type fatty acid-binding protein; Cardiac troponin T; Renal failure.

HEART-TYPE FATTY ACID BINDING-protein (H-FABP) was introduced in 1988 as a potential novel biochemical marker for the early diagnosis of acute myocardial infarction (AMI).<sup>1</sup> This was soon confirmed by many other studies.<sup>2-7</sup> Under normal conditions, H-FABP is not present in plasma or interstitial fluid, but is released into the blood upon cardiac cellular injury. The H-FABP is released into plasma within 2 hours after symptom onset and is reported to peak at about 4-6 hours and to return to normal base line level in 20 hours. Within the period of 30-210 minutes after symptom onset, H-FABP has > 80% sensitivity for the diagnosis of AMI.<sup>4-5</sup> Within the interval of 0-6 hours after symptom onset, the other cardiac

markers such as creatine kinase (CK), CK-muscle and brain (MB) (mass or activity), cardiac troponin I (cTnI) and cardiac troponin T (cTnT) will only be starting to accumulate in the plasma, and their sensitivity has been reported to be around 64%.<sup>8</sup> The exact route(s) of excretion of H-FABP from the circulation is not fully understood. As suggested by previous studies, the kidney may be the major route of excretion of H-FABP from circulation.<sup>4,6,9</sup> The effect of disease states and in particular kidney disease on the renal handling of H-FABP has not yet been fully evaluated. The aim of this study was to compare the effect of renal failure on the level of H-FABP and cTnT concentrations.

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## Methods

The study population consisted of a small group of 16 patients with renal failure (6 females, 10 males, aged between 30-70 years) without evidence of acute coronary syndromes. These patients were on routine regular haemodialysis or peritoneal dialysis, and were recruited from the Renal Unit at the Royal Infirmary of Edinburgh, U.K. These patients had a range of diseases that were commonly seen in the renal unit including chronic glomerulonephritis (3 patients), interstitial nephritis (3 patients), adult dominant polycystic kidney disease (2 patients), hypertensive and diabetic nephropathy (8 patients). Peripheral blood samples were collected in white Starstedt Monovette vacutainer tubes by venepuncture. The blood samples (5mls) were taken through a peripheral line (intravascular access). The extracted samples were allowed to clot at room temperature for 1 hour and then centrifuged at 4°C, and the resulting serum was divided into small aliquots and frozen at -70°C until analysis. The H-FABP was analysed by an enzyme linked immunosorbent assay method using commercially available assays (Hycult, Cambridge). The analytical sensitivity of H-FABP, presented as mean  $\pm$ 2SD, was  $0.206 \pm 0.047$ g/L.<sup>10</sup> The cTnT was analysed on Elecsys 2010 using commercial assays (Roche, Germany). Ethical approval was obtained from the local ethical committee and an informed consent was obtained from each patient before beginning the study. The study complies with the Declaration of Helsinki.

## Results

The reference ranges quoted by the manufacturer for cTnT ( $< 0.01$ µg/L) and H-FABP ( $< 5$ µg/L) assays were validated by assaying the normal ranges of 20 healthy blood donors samples (healthy blood donor controls: 10 males and 10 females, mean age SD =  $63.8 \pm 8.01$  years (median = 65 years [range 53-75 years])). The mean  $\pm$ SD concentrations of cTnT and H-FABP were  $0.011 \pm 0.002$ µg/L, and  $6.86 \pm 2.21$  µg/L respectively. The coefficients of variation (CVs) of the assays were always  $< 10\%$ .

The serum urea and creatinine concentration in this group of patients was  $19 \pm 9.6$  mmol/L and  $531.3 \pm 231.2$  µmol/L respectively. H-FABP was increased in all 16 patients (mean =  $81 \pm 53.3$ µg/L [range 24-173µg/L]). The cTnT was increased  $\geq 0.1$ µg/L in

8 patients (50%),  $\geq 0.2$ µg/L in 5 patients (31.3%), and  $\geq 0.3$ µg/L in 1 patient (6%). In the remaining 8 patients, cTnT was  $0.069 \pm 0.023$ µg/L (range 0.021-0.087µg/L). There were no correlations between the concentration of either urea or creatinine and the concentration of H-FABP and cTnT. There was a positive correlation between the concentrations of cTnT and H-FABP ( $r = 0.569$ ,  $p < 0.02$ ).

## Discussion

H-FABP is a small (15 Kda) soluble non-enzyme protein. It is composed of 132 amino acids. It is one of the most abundant proteins in the heart and comprises 5-15% of the total cytosolic protein pool in the aqueous cytoplasm. H-FABP exists in high concentrations in the heart only. However, this protein is not totally cardiac specific and occurs in other tissues although in much lesser concentrations.<sup>11</sup> It is present in skeletal muscles in concentrations varying between 0.05-0.2mg/g wet weight of tissue, depending on the muscle fibre type studied.<sup>2</sup> It has also been reported in very low concentrations in tissues like the kidney, aorta, testes, mammary glands, placenta, brain, adrenal glands, adipose tissue, and stomach.<sup>11</sup> Preliminary but promising applications of these proteins have been demonstrated for liver rejection, viability selection of kidneys from non-heart-beating donors (NHBD), inflammatory and ischaemic bowel disease, traumatic brain injury and in the prevention of muscle injury in trained athletes.<sup>12</sup> The cytoplasmic to vascular concentration of H-FABP is of the order of 200,000:1. The plasma or serum concentration of H-FABP under normal conditions is  $< 5$ µg/L.<sup>1</sup> This makes the plasma estimation of H-FABP a suitable indicator for the early detection and quantification of myocardial tissue injury. A rise in serum and urine H-FABP concentrations above normal values is seen in patients presenting with AMI as early as 1.5 hours after symptom onset.<sup>6</sup> Studies in animals have also shown decreased myocardial tissue content and rising plasma and urine concentrations of H-FABP very early after coronary artery ligation.<sup>9</sup> Some of the more recent studies have questioned the value of these early markers (H-FABP and myoglobin) when compared with specific markers like cTnI.<sup>13</sup>

The reason for elevated H-FABP in our study of patients with chronic kidney disease (CKD) on

regular dialysis is not clearly understood. These patients did not have any evidence of myocardial injury clinically (neither by history nor by electrocardiogram criteria). This may simply be due to lack of clearance by the diseased kidneys, because H-FABP is totally cleared by the kidneys. Uraemic cardiomyopathy is a recognised complication of CKD and it may be possible that some of the leakage of H-FABP could reflect sub-clinical myocardial injury. The release of H-FABP may be from other tissues containing this protein which are affected by CKD or by drugs used in management e.g. brain or bowel. H-FABP circulates for a longer time (> 25 hours) after AMI in the presence of renal failure.<sup>4</sup> Gorski *et al.* reported that H-FABP and myoglobin concentrations were both significantly elevated in patients with renal failure. The concentrations of these markers were not affected by dialysis. In addition, the myoglobin to H-FABP ratio in this group was similar to the ratio found in patients with myocardial infarction.<sup>14</sup>

The cTnT (34 KDa) was first introduced in 1989 as a marker for AMI. The upper limit for cTnT has been reported as < 0.1µg/L, but concentrations between 0.03-0.1µg/L may also have significance as markers of an adverse outcome.<sup>15</sup> This marker appears in the serum within 12 hours after symptom onset in patients with AMI. Elevated cTnT in patients with unstable angina is associated with poor prognosis.<sup>16</sup> The increase correlates well with the severity of coronary artery lesions as determined by angiography. The cTnT marker is a sensitive and specific marker of myocardial injury. However, elevated cTnT concentrations have been reported in a significant numbers of patients with chronic renal failure. These levels do not seem to be affected by haemodialysis, with elevations persisting after treatment.<sup>17-18</sup> Nadheem *et al.* have shown that cTnT had lower sensitivity and specificity for the diagnosis of AMI compared with cTnI in patients with CKD on dialysis. They also demonstrated that increased cTnT was associated with all causes mortality during two years follow-up.<sup>19</sup> A significant numbers of patients in our study had elevated cTnT concentrations. These elevated levels may be due to a combination of slow clearance and/or uraemic cardiomyopathy.

In a separate study, we used the following concentrations to define the presence of AMI: H-FABP ≥ 12.5µg/L (sensitivity = 91.4%, specificity

= 86%); cTnT ≥ 0.1µg/L (Sensitivity = 76.4%, Specificity = 99%). These cut-off concentrations for the diagnosis of AMI were based on receiver operating characteristics (ROC) analysis between patients with AMI (n = 45 patients) and patients without AMI (stable angina, atypical angina, normal controls, n = 70 patients).<sup>20</sup> Based on these cut-off values, all 16 patients would meet the criteria for the diagnosis of AMI if we used H-FABP concentrations. Also significant numbers (8 patients, 50%) had raised values ≥ 0.1µg/L.

## Conclusion

This study indicates that the efficiency of H-FABP for the diagnosis of AMI in the presence of renal failure is limited. The presence of renal failure may also interfere with the specificity of cTnT for the detection of myocardial injury. This interference should be borne in mind when interpreting the result of these two markers in patients with acute coronary syndromes in the presence of renal impairment.

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