Metabolic profile of pericardial fluid of congenital and acquired heart disease patients and their comparison with serum using 1H NMR spectroscopy

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INTRODUCTION: Pericardial Fluid (PCF) is a pale yellow serous fluid present in the pericardial cavity which is believed to be a transudate generated by the net result of hydrostatic pressure and osmotic gradient between plasma and PCF [1]. A study involving small group of 11 patients undergoing open-heart surgery reported the levels of protein and electrolyte in PCF [2], but the presence of other small molecular weight metabolites and lipids, and their concentration levels are not reported any where to the best of our knowledge. Two studies focused on pathologic effusions, each of them obtained normal PCFs during open-heart surgeries in a small number of patients. However, neither of them reported the composition of the normal PCF [3, 4]. The composition of normal PCF in patients undergoing open heart surgery using biochemical and hematologic parameters has been reported [5]. Biochemical and hematologic tests are often suggested on the PCF after a therapeutic or diagnostic pericardiocentesis. The results at times are interpreted according to Light's criteria for pleural effusions [6], However, this criterion may not be useful in the case of pericardial effusions as reported [5, 7]. Since PCF is a site-specific fluid, its metabolic composition may alter in various diseases affecting pericardium such as pericardial effusion and pericarditis. However, to study the metabolic changes in PCF in diseases conditions, first we should know its normal metabolic composition. ^{1}H NMR spectroscopy has been successfully applied for the study of various body fluids [8]. However, the metabolic profile of PCF has not been reported. The present study focuses on the first application of ^{1}H NMR spectroscopy to human PCF obtained from the patients undergoing open-heart surgeries, aiming at identifying many metabolites under normal conditions.

MATERIALS AND METHODS: Patients being investigated/routinely managed for open-heart surgery (due to congenital, coronary or valvular disease) attending the Department of Cardiovascular and Thoracic Surgery in SGPGIMS, India were candidates for this study. Standard protocols of recording of detailed history, clinical evaluation, baseline biochemical and hematological tests and ethical procedures were followed. The exclusion criteria for the patients were prior myocardial infarction within 3 months, any known pericardial disease, or the use of medications associated with pericarditis. About 1mL PCF specimens were taken and arterial and venous blood samples were also simultaneously withdrawn. PCF specimens were snap-frozen in liquid nitrogen; kept in dark and stored at -80ºC, until ^{1}H NMR experiments were performed. Blood specimens were collected in a serum separator tube and kept for 15 minutes to allow clotting. Thereafter, separated serum was collected in a clean tube and again centrifuged for 5 min at 8,000 rpm and frozen in cryogenic vial at -80ºC until NMR experiments were performed.

For ^{1}H NMR analysis, PCF specimens were centrifuged at 4000 *g* for 10 min at 4ºC. One-dimensional ^{1}H NMR experiments viz. single pulse with presaturation and CPMG with presaturation for both PCF and serum specimens were performed using standard parameters. Lipid extraction was performed on 0.5mL PCF and serum specimens with the extraction method reported earlier [9]. Afterwards all the dried and lyophilized lipid extract samples were re-dissolved in 500µL $CDCl_3$ (obtained from Sigma-Aldrich) and single pulse ^{1}H NMR experiments were recorded.

RESULTS: A total of 107 patients were included in the study of which 62 were adults and 45 pediatric patients. In the adult patients [median (range) age 34 (19-65) years; 39 males], specimens were obtained from three categories (1) valve replacement (n=44), (2) congenital heart diseases (n=7) of which 5 patients had atrial septal defect (ASD) and two patients had ventricular septal defect (VSD) and (3) coronary artery bypass graft (n=11). The specimens of pediatric patients [median (range) age 6 (2-15) years; 32 males] included both cyanotic (n=14) and acyanotic (n=31) congenital heart disease patients. The etiology of all the cyanotic patients was Tetralogy Of Fallot (TOF) type. The aetiologies of acyanotic patients were: (1) ASD (n=15), (2) VSD (n=15) and total anomalous pulmonary venous return (TAPVR) (n=1).

Fig. 1 shows typical ^{1}H NMR spectra of human PCF from a patient with valve replacement obtained using single pulse sequence (Fig. 1A) and CPMG pulse sequence (Fig. 1B). The unambiguous assignments of various resonances were made from two-dimensional DQF-COSY and TOCSY spectra. No significant difference was observed in PCF metabolites between two categories of pediatric patients. Similarly there was no difference among the three groups of adult patients. In a comparison of metabolites between the two groups of pediatric and adult patients, it was observed that alanine, lysine, succinate, citrate and histidine were significantly more and acetone, glucose and formate were significantly less in PCF of adults as compared to pediatric patients (Fig. 2).

Assignment and quantification of the metabolites was also performed in the serum specimens of all the patients. The concentrations of lipids were observed to be lesser in PCF than in sera in both pediatric and adult patients. However, the concentrations of small molecular weight metabolites such as alanine, lysine, glutamine, citrate, histidine, tyrosine and formate were more in PCF as compared to sera (CPMG spectrum) with the exception of lactate (Fig. 3). No significant differences were observed between the metabolites of arterial and venous serum specimens in pediatric as well as adult patients. After lipid extraction, the quantitation was done for the lipid metabolites viz. total cholesterol which includes cholesterol (CHOL) and cholesterol ester (CHOLest), free cholesterol, cholesterol ester, phospholipids (PL) and triglycerides (TG). All the quantitated lipid metabolites were significantly less in PCF as compared to those in sera (Fig. 4).

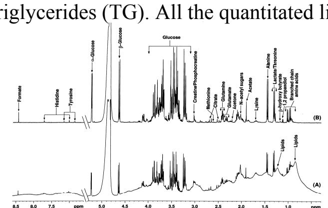

Fig.1: Typical ^{1}H NMR spectra of human PCF.

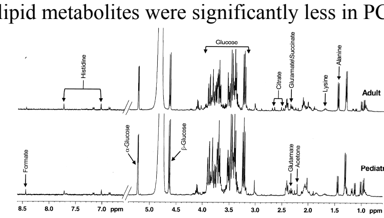

Fig.2: Typical ^{1}H NMR spectra of PCF of a pediatric and an adult patient.

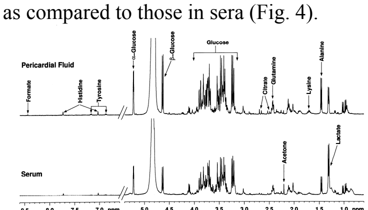

Fig.3: Typical ^{1}H NMR spectra of PCF and serum of an adult patient.

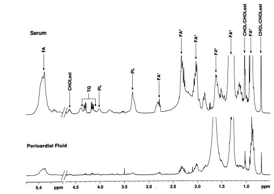

Fig.4: Typical ^{1}H NMR spectra of lipid extract of PCF and serum. FA; fatty acids

DISCUSSION: In the PCF of both pediatric and adult patients common metabolites were observed in different categories. However, there was a significant difference in the concentrations of metabolites between pediatric and adult patients. These differences could be related to be age dependent. Changes in the concentration of various metabolites with age are very well reported using urine specimens [10, 11]. The concentration of PCF metabolites reported in pediatric and adult groups could serve as reference ranges in the patients with both the age groups. However, definitive biological roles linking these metabolites with age are yet to be established.

PCF specimens of pediatric and adult patients were compared with their arterial and venous serum specimens. This is in accordance with earlier studies where, for the diagnosis of pericardial effusions, the biochemical and hematological data of PCF were compared with the data of plasma and serum specimens [4, 5]. This study showed that PCF specimens which are the transudates of serum vary in lipid as well as in small molecular weight metabolites composition as compared to the serum. The concentrations of various metabolites represent the normal concentration ranges in PCF. A word about the source of the PCF specimens may be worth mentioning. For obvious ethical reasons, fluid was obtained from patients undergoing open-heart surgery, rather than from healthy volunteers. The results may therefore be affected by circumstances such as anesthesia, or the presence of comorbid conditions, or the chronic underlying cardiac condition itself. However, the prospective nature of the study and large sample size shows the potential for generalizing the results to the normal population which is supported by the lack of correlation between fluid quantitative analysis with various patients' characteristics (type of surgery and comorbid conditions), as well as by the inclusion of only clinically stable patients who did not have evidence of pericardial disease. This analysis may provide a baseline for better understanding of pericardial fluid physiology, potentially enabling a more comprehensive and accurate analysis of the compositional variations related to pericardial diseases.

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