

Original Article

Analysis of early predictor effect of serum Glutathione S-Transferase P1 isoenzyme on post-operative results in paediatric cardiac surgery

Ugur Gocen, Vecih Keklik, Atakan Atalay, Hafize Yaliniz, Orhan Kemal Salih

Department of Cardiovascular Surgery, Medical Faculty of Cukurova University, Adana, Turkey

Received February 24, 2016; Accepted May 10, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: *Background:* Glutathione S-Transferase P1 is an important enzyme playing role in detoxification of oxidative stress products developing in the course of inflammation. *Aim:* The aim of this study was to detect if Glutathione S-Transferase P1 (GSTP1) isoenzyme might be an early predictor of mortality. *Methods:* This prospective study examined 30 paediatric patients to have had open cardiac surgery under cardiopulmonary bypass. Patients were divided into two groups; those discharged without any problem (Group 1, N=23) and exitus (Group 2, N=7). Blood samples were collected at five times of (i) after anesthesia induction (T1), (ii) 10 minutes after cardiopulmonary bypass started (T2), (iii) 10 minutes after cross clamp was placed (T3), (iv) at the 30th minute following cardiopulmonary bypass (T4) and (v) at the 8th hour following the operation (T5). *Results:* Group 1 patients had been discharged from hospital and 7 of them died (Group 2). In T1, a statistically significant difference (two folds) was detected between the two groups in terms of GSTP1 values. Although GSTP1 values of both groups decreased during cardiopulmonary bypass period (T2, T3), a statistically significant difference was found between the two groups ($p=0.001$). GSTP1 values of the study groups in T4 and T5 were measured to be higher than the values recorded in T1, T2 and T3 ($P0.001$). GSTP1 values of the Group 2 were also detected to increase by two folds in T4. Comparison of creatinine values produced no statistically significant difference between the two groups in the period from T1 to T4. Mean creatinin level of Group 2 was observed to considerably increase in T5 and a statistically significant difference was observed between the creatining levels of the two groups ($P0.001$). *Conclusion:* GSTP1 isoenzyme may be used as an early prognostic marker following a pediatric cardiac surgery with CPB.

Keywords: Paediatric open cardiac surgery, cardiopulmonary bypass, Glutathione S-Transferase P1 isoenzyme, creatinine, prognosis

Introduction

In paediatric cardiac surgery, there are limited number of studies making a comparison of the relationship between inflammation and cardiopulmonary bypass, on one hand, and mortality and morbidity evaluation, on the other hand. GSTP1 belongs to multigene isozyme family involved in cellular response to oxidative stress and apoptosis. Initial retrospective proteomic analyses made in the scope of the present study suggested GSTP1 to be associated with heart failure [1]. This study evaluated the relationship between the values of GSTP1 isoenzyme, one of the inflammation enzymes with cardiac significance, and the mortality and morbidity of the patients to have undergone con-

genital open cardiac surgery. This study is important in terms of being the first literature study addressing this issue in the context of pediatric heart surgery.

Oxidative stress and inflammation are emerging as unifying pathophysiological mechanisms underlying cardiovascular disease [2-4]. There are various cellular detoxification systems which provide protection against both endogenous and environmental noxious substances. In particular, the superfamily of glutathione S transferases is associated with the regulation of inflammation through modulation of prostaglandin signaling pathways and oxidative stress and [5] through regulation of normal cellular physiology [6, 7].

Glutathione S-Transferase P1 is an early predictor

Organ damage after cardiac surgery with cardiopulmonary bypass results from two related pathophysiologic mechanisms: systemic inflammatory response syndrome and ischemia/reperfusion injury [8]. Systemic inflammatory response syndrome is triggered by the exposure of blood to large areas of synthetic materials of the extracorporeal circuit. It causes a complex inflammatory reaction, involving activation of complement, platelets, neutrophils, monocytes, and macrophages with increased blood concentrations of cytokines and leukotrienes. In addition, systemic inflammatory response syndrome initiates activation of the coagulation, fibrinolytic, and kallikrein cascades. A subsequent increase in endothelial cell permeability allows transvascular migration of activated leukocytes into the tissues exposed to additional vascular and parenchymal damage [9, 10].

Ischemia/reperfusion injury is triggered mainly in the heart and lungs, secondary to aortic cross-clamping and cardioplegic arrest [11, 12]. During aortic crossclamping, heart is excluded from the circulation to protect it from cardioplegia and hypothermia. Lungs are deprived as well of pulmonary blood flow. Ischemia/reperfusion injury has been documented also in other organs, such as kidneys and intestines, probably due to alterations in blood flow at microcirculatory level [13, 14].

GSTP1 is the most prevalent mammalian isozyme of the Glutathione S-transferase family. GSTP1 is an important regulator of inflammation and plays a key role in cellular homeostasis, including inhibition of apoptosis, detoxification of reactive oxygen species, and maintenance of cellular redox state [15-17]. Results of previous studies revealed that it has a better correlation with failure grade than pro-Brain natriuretic peptide in chronic heart failure.

This study aimed to evaluate the relationship with morbidity and mortality of the GSTP1 isozyme as an early predictor during and after congenital cardiac surgery.

Material and methods

Patients

This prospective study included 30 patients who had undergone open heart surgery with

cardiopulmonary bypass due to congenital heart disease. After receipt of approval from the hospital ethics committee and written informed consent from the patients, those patients who had been scheduled for the first-time congenital heart surgery were studied. Patients who were going to undergo open heart surgery for the first time and patients whose pre-operative kidney functions, liver functions, cerebral and cardiac functions were normal (Ejection fraction > 50%) were included in the scope of the present study. Patients who would need to have re-operation, had an additional disease and whose vital findings were instable were excluded from the study. Ages of patients varied in 2 months - 150 months year range (Group 1 - 46.26 ± 41.584 and Group 2 - 29.29 ± 29.75 on average).

Method of anesthesia

Anesthesia was administered according to a fixed protocol [18]. Premedication consisted of intravenous midazolam, administered as 0.1 mg/kg preoperatively in pre-op patient room. General anesthesia was induced by sufentanil, 2.5 g/kg, and midazolam, 0.1 mg/kg. Tracheal intubation was achieved with pancuronium, 0.1 mg/kg, and the lungs were ventilated with air and oxygen (fraction of inspired oxygen-0.4). Arterial catheter was placed into the radial or brachial arteries. A flow-directed central venous catheter was inserted into the right internal jugular vein, and an indwelling bladder catheter was used for urine collection. Anesthesia was maintained with sufentanil, midazolam, and pancuronium. Cefuroxim, 50 mg/kg, was administered after induction. Hydroxyethyl starch, 200/0.56% solution, and lactated Ringer solution were used to obtain a mean arterial pressure of 45 mmHg and to maintain filling pressures and cardiac output. Packed RBCs were transfused at 4.5 mmol/L hemoglobin level. Inotropic support with dopamine was initiated at the cardiac index of 2.2 L/min/m². Diuretics, mannitol and aprotinin were not administered during the entire study period. Patient characteristics and perioperative variables were recorded prospectively.

CPB

Nonpulsatile cardiopulmonary bypass (CPB) was performed using a roller pump (CAPS HLM; Stockert Instruments; Munich, Germany) and a

Glutathione S-Transferase P1 is an early predictor

membrane oxygenator (Cobe Optima; Cobe Laboratories; Lakewood, CO). Extracorporeal circuit was primed using a 6% hydroxyethyl starch with lactated Ringer solution. During cardiopulmonary bypass, the flow was maintained at 2.4 L/min/m² with moderate hypothermia (28-32°C) and pH-stat regulation of blood pH. Cold St. All study participant patients underwent normoxic cardiopulmonary bypass (PaO₂: 80-150 mmHg). Thomas solution was infused into the aortic root to maintain cardioplegia during aortic cross-clamping. During cardiopulmonary bypass, the mean arterial pressure was allowed to vary in 45-60 mmHg range. Deviations were corrected by phenylephrine or nitroglycerine.

Blood sampling was performed after induction of anesthesia (T1), 10 minutes after cardiopulmonary bypass started (T2), 10 minutes after aortic crossclamping (T3), 30 minutes after completion of cardiopulmonary bypass (T4), and 8 hour after operation (T5).

Biomarkers

Glutathione S-Transferase P1: Glutathione S-transferases (GSTs) have three main functions in organism. First function is that GSTs play a role in conjugation- and reduction-based reactions during detoxification process. Conjugation is achieved by two different mechanisms of catalytic and non-catalytic nature. Second function is that GSTs plays an enzymatic role in detoxification process. Another main function is that GSTs act as intracellular carrier protein. In the scope of this function, GSTs, as intracellular equivalent of albumin in plasma, perform the function of bounding many hydrophobic compounds and of achieving their intracellular transmission. And the third main function of GSTs is to prevent induction of organ-specific toxicity by xenobiotics. Various types of GSTs, which are specific to each organ, function as independent isoenzymes in organ-specific toxicity induced by xenobiotics.

For Glutathione S-Transferase P1 (GSPT1) analysis, 2 cc venous blood samples were collected from central catheter into citrated tubes and sent to the laboratory. Enzyme-linked immunosorbent assay (ELISA) for Glutathione S-Transferase P1 (Hepkit-Pi; Biotrin International, Dublin, Ireland) was performed and quantified spectrophotometrically by an automated microplate reader (Anthos, Salzburg, Austria).

Creatinine

Creatinine is anhydride of creatine. It is mainly formed by extraction of phosphate from creatine phosphate inside the muscle in an irreversible and non-enzymatic way. Serum creatinine level testing is useful in routine scanning and prompt monitoring. There is a reverse linear relationship between serum creatinine levels and glomerular filtration speed. In cardiopulmonary bypass, it may display itself by an increase in creatinine levels upon failure of renal clearance due to inflammatory reactions and organ perfusion disorders.

For GSTP1 analyses, 2 cc venous blood samples were collected from central catheter into citrated tubes and sent to the laboratory. Collected samples were analyzed using kinetic Jaffe method. Statistical significance was set at 0.5-1.2 mg/dl.

Statistical analysis

SPSS 19.0 package programme was used in the statistical analysis of study data. Categorical measurements were summarized in number and percentage while numeric measurements were summarized in average and standard deviation (median and minimum-maximum values were presented where necessary). Mann Whitney U test was used to compare two numeric groups with abnormal distribution. Repeated Measurements Analysis was used when the study hypothesis were verified by the comparisons of the changes recorded in the numerical measurements made on the same participants at different times and Friedman test was used when the study hypothesis were not verified by the concerned comparisons. ROC analysis was done to determine the appropriate breakpoints for creatinine and GSTP1 variables. "Sensitivity", "Specificity", "Positive Predictive Value", "Negative Predictive Value" and "Area Remained under the Curve" values were presented for each variable. Statistical significance was set at "0.05" for all tests.

Results

Twenty three (23) patients were discharged in healthy condition (Group 1) and 7 of them died (Group 2). According to inter-group age comparisons, age range of Group 1 was 46.26±41.584 months while age range of Group 2 was 29.29±29.75 months, with no statistically sig-

Glutathione S-Transferase P1 is an early predictor

Table 1. Comparison of variables

	Group 1 (n=23)	Group 2 (n=7)	p
Age (month)	46.26±41.58	29.29±29.75	0.360
Female/Male	8/15 (34.78/65.21%)	4/3 (57.14/42.85%)	0.285
Cyanotic patient/Group	9/23 (39.13%)	5/7 (71.428%)	0.204
CPB time (minute)	69±36.30	91.43±39.23	0.174
Cross-clamp time (minute)	53.63±24.48	90.7±31.71	0.144

CPB: Cardiopulmonary bypass.

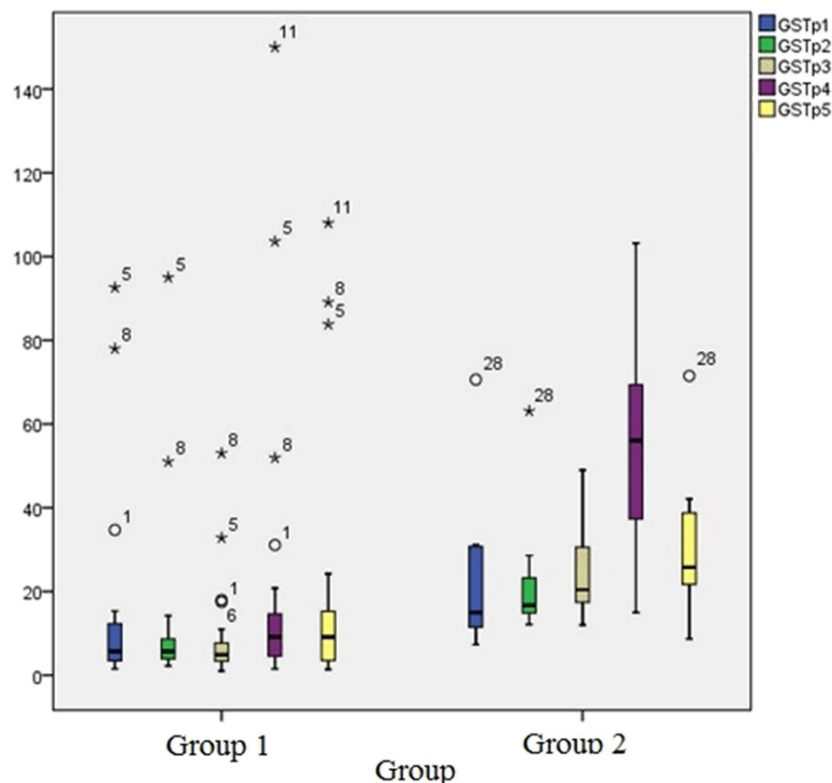


Figure 1. Graphic showing the GSTP1 values of Group 1 and Group 2.

Table 2. T-based evaluation of serum creatinine values

Creatinine (mg/dl)	Group 1 (n=23)	Group 2 (n=7)	p
T1	0.344±0.176	0.302±0.154	0.501
T2	0.323±0.125	0.354±0.164	0.362
T3	0.338±0.128	0.358±0.158	0.326
T4	0.397±0.135	0.378±0.174	0.288
T5	0.378±0.174	0.881±0.368	0.001
p time	0.124	0.009	

nificant difference between the two groups in terms of age range ($p=0.360$) (**Table 1**), Cardiopulmonary bypass ($p=0.174$) and cross

clamp times ($p=0.144$) were found to be statistically insignificant for both groups. Ratios of the cyanotic patients in the study groups were recorded to be statistically insignificant ($p=0.204$).

Examination of the mean GSTP1 values (**Figure 1**) showed that GSTP1 values were in 14.704 ± 23.465 range for Group 1 and in 28.342 ± 22.157 range for Group 2 in T1, pointing out a statistically significant difference between the two groups ($p=0.029$) (**Table 3**, **Figure 1**). GSTP1 values were recorded to be in 12.008 ± 20.647 range for Group 1 and in 24 ± 18.031 range for Group 2 in T2, revealing a statistically significant difference between the two groups ($p=0.001$) (**Table 3**, **Figure 1**). GSTP1 values were observed to be in 9.160 ± 11.89 range for Group 1 and in 25.342 ± 12.697 range for Group 2 in T3, underlying a statistically

significant difference between the two groups ($p=0.001$) (**Table 3**, **Figure 1**). Mean GSTP1 values were found to be in 21.239 ± 35.831 range for Group 1 and in 55 ± 28.760 range for Group 2 in T4, producing a statistically significant difference between the two groups ($p=0.001$) (**Table 3**, **Figure 1**). GSTP1 values were recorded to be in 19.752 ± 30.100 range for Group 1 and in 32.414 ± 20.362 range for Group 2 in T5, pointing a statistically significant difference between the two groups ($p=0.016$) (**Table 3**, **Figure 1**).

T-based comparison of GSTP1 values of Group 1 produced no statistically significant difference between T1 and other periods ($p=0.112$)

Glutathione S-Transferase P1 is an early predictor

Table 3. Comparison of serum GSTP1 values

GSTP1	Group 1 (n=23)	Group 2 (n=7)	p
T1	14.704±23.465	28.342±22.157	0.029
T2	12.008±20.647	24±18.031	0.001
T3	9.160±11.89	25.342±12.697	0.001
T4	21.239±35.831	55±28.760	0.001
T5	19.752±30.100	32.414±20.362	0.0016
p time	0.112	0.04	

GSTP1: Glutathione S-Transferase P1 isoenzyme.

Table 4. Comparison of T1 and T4 GSTP1 values of Group 2 patients

	T1	T4	p
Group 2 GSTP1	28.342±22.157	55±28.760	0.001

GSTP1: Glutathione S-Transferase P1 isoenzyme.

Table 5. Comparison of T's showing significant difference between Group 1 and Group 2

	Cut-off	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
GSTP1-T1	7	100	56	41	100
GSTP1-T2	10	100	78	58	100
GSTP1-T3	11	100	83	64	100
GSTP1-T4	15	86	78	54	95
GSTP1-T5	8	100	48	37	100

GSTP1: Glutathione S-Transferase P1 isoenzyme.

(**Table 3**) while T-based comparison of GSTP1 values of Group 2 revealed no statistically significant difference between T1 and other periods except T4 (**Table 3**). In T4, GSTP1 value of Group 2 was recorded to increase at a statistically significant level compared to T1 ($p=0.001$) (**Table 4**).

Examination of the ROC analysis results of the variables found to be statistically significant in Group 1 and Group 2 revealed 86% sensitivity and 78% specificity; 54% positive predictive value and 95% negative predictive value when cutoff value was taken as 15 ng/dl for GSTP1 values in T4 (**Table 5**). Regarding GSTP1 values, the area remained under the ROC curve was calculated to be 0.879 in T4 and 0.801 in T5 (**Table 5, Figure 2**).

Initial GSTP1 values (T1) were higher than cardiopulmonary bypass values (T2 and T3) in both Groups (**Table 3**). After cardiopulmonary bypass (T4 and T5), GSTP1 values were record-

ed to increase in Group 1, not up to very high values though. Concerned increase was suggested to be caused by reperfusion-induced inflammation. After initiation of cardiopulmonary bypass, higher GSTP1 values were observed (**Table 3**) in mortality group (Group 2), which suggests that it is meaningful to use GSTP1 values as an early marker.

Discussion

Body produces energy by burning carbohydrates and fat; in other words, oxidation must take place for energy generation. These metabolic reactions normally result in the formation of free oxygen radicals, which play a role in defending the body against foreign substances and infectious agents [19, 20]. The body maintains a balance between various pre-oxidative factors affecting the system and the antioxidant system developed to counteract these factors. Unlike healthy individuals, patients with congenital heart disease fail to meet the biological needs of tissues due to ischemia, reperfusion, and chronic hypoxia and, in turn, they are exposed to excess oxygen radicals, which deteriorate patient's condition and result in treatment difficulties [21].

It is known since the start of the use of cardiopulmonary bypass method in heart surgery that this procedure induces systemic inflammatory response syndrome [22, 23]. Blood contact with a foreign surface during cardiopulmonary bypass, ischemia and reperfusion damage, temperature changes recorded in cooling and heating periods and duration of these periods, endotoxins and finally pro-inflammatory agents developed by operative trauma might result in irregularities related to pulmonary, neurological and/or coagulation systems which are together called "systemic inflammatory response syndrome (SIRS)".

Despite the developments in anesthesia, surgery and perfusion techniques; inflammatory response and SIRS in cardiopulmonary bypass operations still constitute an important reason for morbidity and mortality. This may cause serious organ failures and even death especially among children whose immune system development has not been completed yet. However,

Glutathione S-Transferase P1 is an early predictor

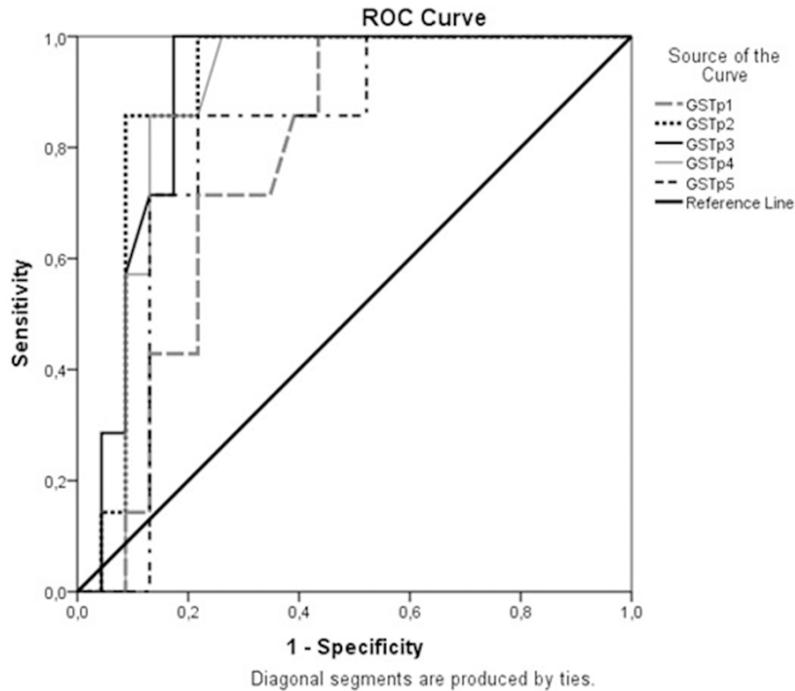


Figure 2. Graphic showing the area of GSTP1 values remaining under the ROC curve.

search still continues for a reagent which has a stronger relation with mortality and morbidity and which can be detected at an earlier period than the existing reagents. Since oxygen radicals develop at higher levels during hyperoxic cardiopulmonary bypass, normoxic (PaO₂ 80-150 mmHg) cardiopulmonary bypass protocol was followed in the scope of the present study. Therefore, both the patients involved in the study were tried to be protected from oxidative stress and Glutathione S-Transferase P1 enzyme values of all cases were evaluated via normoxic cardiopulmonary bypass.

In both adult and paediatric cardiac surgery, many detrimental effects of cardiopulmonary bypass on end organ dysfunction were previously believed to be mediated by activation of inflammatory response [2, 20]. It might be expected that cardiopulmonary bypass-related systemic inflammatory response syndrome and multiorgan injury will be enhanced during normothermia since most enzymatic processes occur optimally at 37°C. This suggestion is supported by the clinical studies conducted on adults [21] to compare normothermic cardiopulmonary bypass (35°C-37°C) with hypothermic cardiopulmonary bypass (28°C-30°C) to

prove that the former is associated with significantly elevated levels of inflammatory markers. Inflammation is known to decrease during hypothermia, compared to the normothermic period in cardiopulmonary bypass. Glutathione S-Transferase P1 levels were observed to decrease during cooling period compared to basal levels in cardiopulmonary bypass. This situation might be related to the decrease in inflammation and enzymatic activity as a result of hypothermia. After warming period, at the 30th minute following the bypass, GSTP1 levels increased more than two times in relation to the increase in inflammation and enzyme activation. Superoxide radicals incre-

ase and lead to an increase in GSTP1 enzyme activity in connection with the stimulation of inflammation.

Present study revealed numeral differences in GSTP1 values, including pre-operative values, between Group 1 and Group 2 and such differences were found to be statistically significant in all T's. GSTP1 values of the samples collected during pumping (T2, T3) were recorded to decrease in both groups, producing a statistically significant difference between the two groups (**Table 3**) (p=0.029). A two fold increase was observed at the 30th minute (early post-operative period following completion of cardiopulmonary bypass) (T4) compared to T1 in Group 2. It was also determined that there was a statistically significant difference in creatinine levels between the two groups at the 8th hour post-operation (**Table 2**). In the light of the present study, it can be suggested that changes in GSTP1 enzyme levels are more valuable as an early mortality and morbidity indicator.

Comparison of GSTP1 values showed a statistically significant difference between the two groups in all T's except T1. It is notable that there is a statistically significant difference in

Glutathione S-Transferase P1 is an early predictor

basal values in T1. Related studies show that total oxidant level, total anti-oxidant level and oxidative stress indices of children with cyanotic congenital heart disease are statistically significantly higher than acyanotic patients [24]. Reason of the statistically significant difference in T1 is the high number of cyanotic patients in Group 2. Blood and tissue oxygen levels of cyanotic patients are lower compared to acyanotic patients. After corrective surgery, alterations in blood oxygen levels are more significant in cyanotic patients. As a result, GSTP1 enzyme activity increases more in cyanotic patients. This situation explains the higher preoperative, perioperative and postoperative GSTP1 levels in Group 2 containing higher number of which cyanotic patients (**Table 3**).

Mean GSTP1 levels recorded to be higher in Group 2 than Group 1 in T1 can be a criterion in predicting the poor prognosis of these patients. GSTP1 values decreased in both groups during cardiopulmonary bypass (T2, T3), producing statistically significant difference (**Table 3**). Compared to T1, two-fold increase was recorded at the 30th minute after cardiopulmonary bypass (T4) in Group 2. It is thought that GSTP1 values of both groups increased in T4 and T5, secondary to inflammation response to ischemia-reperfusion. GSTP1 levels which were recorded to be higher in Group 2 than Group 1 indicate that GSTP1 can be used as an early predictor of poor prognosis.

Over the last few years, the prognostic importance of mildly elevated pre-operative serum creatinine levels or small increases in post-operative creatinine levels has become more obvious, leading to a shift in the general approach towards the importance of even low degrees of acute kidney injury. A mild elevation (1.3-2.0 mg/dL) of the pre-operative creatinine level significantly increases the probability of peri-operative mortality, low cardiac output, haemodialysis, and prolonged hospitalization. Potential reasons for renal dysfunction include cardiovascular compromise, prolonged cardiopulmonary bypass time, increased catecholamine level, non-pulsatile flow, hypothermia, renal hypoperfusion, and the induction of inflammatory mediators [25]. Numerous factors may collectively contribute to renal ischaemia and systemic inflammatory responses, resulting in generous formation of reactive oxygen

species and depletion of endogenous antioxidants [25]. Serum creatinine values provide data on renal function; however, hospital mortality, period of stay in intensive care unit and hospitalization periods prolong in patients developing renal function disorder in post-operative period. Small increases in serum creatinine levels may be bad prognostic criteria in cardiac surgery; however, it becomes more important to find the early reagents that may be crucial in prognosis as it is difficult to measure such small changes in early periods.

Aim of this study was to show applicability of GSTP1 isoenzym as an early reagent for mortality in patients to have undergone congenital heart surgery. In the light of this aim, the authors of the study attempted to make a simple study which would be a good example for future studies and may provide an insight to protective treatment protocols against inflammatory system. This study showed that rate of mortality development is statistically significant for patients whose GSTP1 value keeps increasing during cardiopulmonary bypass and in the post-operative period (**Table 3**).

Study limitations and clinical Implications

The single-center nature of this study, although prospective, is a limitation. Another limitation of the present study was the relatively small size of the study groups.

Conclusions

Even though the study was carried out on a small group of patients, a statistically significant difference was recorded between the GSTP1 enzyme levels of the two groups. As a result of the study, a statistically significant difference was recorded between the GSTP1 levels of the two groups after start of cardiopulmonary bypass (T2). It was also found as a result of this study that high GSTP1 levels early predict mortality during and after congenital heart operations. Therefore, adverse effects of oxidative stress have an influence on prognosis. In addition, early identification of high-risk patients and more-advanced treatment options is of vital importance. Present study hypothesized that GSTP1 levels might be a useful prognostic serum marker for congenital heart patients.

Disclosure of conflict of interest

None.

Address correspondence to: Ugur Gocen, Cukurova Universitesi Tip Fakultesi Kalp ve Damar Cerrahisi, Adana 01330, Turkey. Tel: +90 03223386793; Fax: +90 03223386900; GSM: +90 5055033009; E-mail: ugurgocen@hotmail.com

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