Research Article

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New pathway in rheumatic mitral valve disease: cytochrome P450 and glutathione S transferase isozyme expression

Romatizmal Mitral Kapak Hastalığında Yeni Yolak: Sitokrom P450 ve Glutatyon S Transferaz İzozim Ekspresyonu

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Abstract

Objective: Cytochrome P450 (CYP)1A1, glutathione S-transferase pi (GSTP1) and omega (GSTO1) isozymes were evaluated and compared in patients with the diagnosis of rheumatic mitral valve disease and ischemic mitral valve insufficiency to find out the relationship of the oxidative stress with rheumatic mitral valve disease.

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Materials and methods: The control group consisted of patients operated on due to ischemic mitral valve insufficiency (group I, n:14) while study group consisted of the patients operated on with the diagnosis of rheumatic mitral valve disease (group II, n:29). Mitral valve materials were stained with hematoxylin and eosin. CYP1A1, GSTP1, and GSTO1 immunohistochemical markers were studied.

Results: 20.7% of GSTP1 isozyme protein expression was seen in the study group; however, no expression was detected in the control group. This finding was statistically significant in terms of GSTP1 isozyme. No statistically significant differences in the level of GSTO1 and CYP1A1 protein expression between the study and control groups were observed.

Conclusion: In this study, we found out that GSTP1 isozyme may be related to rheumatic mitral valve disease. A strategy that would help prevent oxidative stress in patients with rheumatic mitral valve disease can be a so valuable means to affect disease progression.

Keywords: Rheumatic mitral valve disease; Ischemic mitral valve insufficiency; GST isozyme expression; Cytochrome P450; Glutathione S-transferase.

Öz

Amaç: Romatizmal mitral kapak hastalığı ve iskemik mitral kapak yetmezlikli hastalarda Sitokrom P450 (CYP)1A1, glutatyon S-transferaz pi (GSTP1) ve omega (GSTO1) izozimleri karşılaştırılarak, romatizmal mitral kapak hastalığı ile oksidatif stres arasındaki ilişki araştırıldı.

Gereç ve Yöntem: Kontrol grubunu, iskemik mitral kapak yetmezliği sebebiyle ameliyat edilen (group I, n:14),

çalışma grubunu ise romatizmal mitral kapak hastalığı nedeni ile ameliyat edilen (group II, n:29) hastalar oluşturmuştur. Mitral kapak materyalleri hematoksilen ve eozinle boyandı. CYP1A1, GSTP1, ve GSTO1 immünohistokimyasal belirteçler ile çalışıldı.

Bulgular: Çalışma grubunda %20.7 GSTP1 izozim protein ekspresyonu gözlemlendi; fakat kontrol grubunda ekspresyon izlenmedi. Bu bulgu GSTP1 izozim açısından istatistiksel olarak önemli bulundu. Çalışma ve kontrol grupları arasında GSTO1 ve CYP1A1 protein ekspresyonları açısından istatistiksel anlamlı farklılık görülmedi.

Sonuç: Bu çalışmada GSTP1 izozimi romatizmal mitral kapak hastalığı ile ilişkili olabileceği görüldü. Romatizmal mitral kapak hastalığı olan hastalarda oksidatif stresi önleyen strateji, hastalığın ilerleyişini engellemek için çok değerli bir seçim olabilir.

Anahtar kelimeler: Romatizmal mitral kapak hastalığı; İskemik mitral kapak yetmezliği; GST izozim ekspresyonu; Sitokrom P450; Glutatyon-S-transferaz.

Introduction

When the mitral valve is examined anatomically and physiologically, it is seen that it is one of the most complex structures of the heart.

Cellular mechanisms of mitral valve dysfunction that are effective in the etiopathogenesis of the disease are not fully known yet. Although there are many studies associated with the etiopathology of rheumatic valve diseases, research on oxidative enzymes and their relationship with rheumatic valvular disease has been done by us.

Although the degenerative valve disease more commonly is observed in developed countries, rheumatic valve disease is detected more commonly in Turkey. 40–65% of adults with rheumatic disease of mitral valve experienced rheumatic fever [1, 2]. After rheumatic heart disease, 50% mitral valve, 30% mitral and aortic valve, and 15% isolated aortic valve were affected [3].

When inflammation seen in acute rheumatoid arthritis (ARA) affects the endocardial tissue, it may cause fibrosis, thickening, calcification and subsequent commissural fusion in the heart valves. Commissural fusion leads to narrowing of the primary orifice area and chordal fusion leads to narrowing of the secondary orifice area [4, 5].

After myocardial infarction (MI), ischemic mitral valve insufficiency (IMVI), which can be seen in 10.9%–19.4%, may develop for various reasons (affects the subvalvular and ventricular structures) [6].

Progressive pulmonary and systemic congestion may occur in 60–70% of untreated mitral valve patients,

20–30% of them experienced systemic embolism, pulmonary embolism may occur in 10%, and infection may be faced in 1–5% [4, 7].

Cardiac oxidative metabolism has been reported to be responsible for heart failure and dysfunction [8]. Mitochondrial dysfunction may develop during ventricular remodeling in rheumatic mitral heart valve diseases [9]. It has been shown that the oxidative metabolism of both dilated and ischemic cardiomyopathy patients is impaired and the ventricular oxidative metabolism disorder is the same in both cardiomyopathies [10]. No difference was found between plasma oxidative properties and oxidative stress indices in patients with degenerative and rheumatic valve illnesses [11]. Total antioxidant capacity of rheumatic heart valve tissue was found to be lower than normal subjects [11]. Oxidized molecules whose effects are neutralized by antioxidants often form new radicals.

In a recent study; valve calcification due to rheumatic heart disease has been related to inflammatory events and an increase in osteoblastic markers [12].

Oxidizing agents such as membrane-related superoxide (O_2^-) and hydrogen peroxide (H_2O_2) cause oxidative stress and induce tissue damage. Oxidative stress results from a series of reactions caused by Reactive Oxygen species [13, 14].

Phase I and Phase II enzyme groups, which are intracellular metabolic antioxidants, play an important part in enzymatic antioxidative mechanisms and in the reversal of the harmful effects of endogenous and/or exogenous xenobiotics and their excretion [15, 16]. Phase II reactions combine the polar metabolites produced by phase I reactions. Cytochrome P450 monooxygenases (Cytochrome P450/CYP450), which constitute an important part of phase I reactions, are effective on oxidation reactions, while Glutathione S transferase (GST) is an essential component of phase II reactions.

Given the roles of these two systems on metabolism, xenobiotics are either less reactive and excreted from the body through phase I enzymes such as CYP1A1 and CYP1B1, or converted into reactive metabolites via phase I enzymes such as GSTO1 and GSTP1 [17–19]. At that point, if the reactive metabolites cannot be conjugated by another enzyme system, they can cause problems like tissue and cell damage. Phase II enzymes conjugate and implement excretion of reactive metabolites produced by phase I reactions to prevent problems such as cell and tissue damage or cancer [20].

Variabilities in the components of phase I and phase II enzyme schemas that are the main pathways of detoxification, antioxidation, and drug metabolism, have not been studied in the etiopathogenesis of rheumatic and degenerative mitral valve diseases. In this context, we

aim to study the expression of GSTO1, GSTP1 and CYP1A1 immunohistochemically.

Materials and methods

Rheumatic and ischemic mitral valve patients who underwent surgery in the Türkiye Yüksek İhtisas Hospital and Medicana Hospital in Ankara, Turkey, between 2015 and 2017 were included in the research. This research was allowed by the Ethics Committee of Türkiye Yüksek İhtisas Hospital (18.11.2015, Number: 44). The control group (n, 14) was operated on with the etiology of ischemic mitral valve insufficiency (due to chordal rupture, chordal elongation, chordal shortening, posteromedial papillary muscle dislocation, and annular dilatation) while the study group (n, 29) was operated on for rheumatic mitral valve disease. Ischemic mitral valve insufficiency was considered to be a control group by reason of it is a disease of ventricular and not a valvular. It was noted that mitral heart valves of patients in the control group were not affected by rheumatic disease. Patients who experienced rheumatic changes such as calcification, fibrosis, thickening, and fusion in the control group during the operation were excluded. Thus, the control group patients were accepted having no mitral valve rheumatic changes. In both groups of patients, those who received anti-inflammatory treatment before surgery, and those with endocarditis and liver disease were excluded from the study.

Immunohistochemical examination method

Macroscopic sections were obtained from the tissues of the study group (rheumatic mitral valve disease patients) and the control group. Paraffin sections were prepared after fixation with 10% formalin. The sections were stained with hematoxylin, and eosin, then examined under a light microscope. For immunohistochemical (IHC) staining, suitable blocks were chosen. The primary antibodies CYP1A1 1/50, GSTO 1/100 and GSTP 1/100 were applied to sections for IHC staining. IHC evaluations were performed using the light microscope to expose the existence of the staining on the endocardial tissues of mitral valves: no cytoplasmic protein expression is indicated as negative staining, and existence of cytoplasmic protein expression as positive staining.

Statistical analysis

Statistical tests were performed with the SPSS software (Statistical Package for the Social Sciences. version 25.0.

SPSS Inc. Chicago, IL, USA). Categorical variables were presented as number (n) and percentage (%) and continuous were given as mean $\pm$ standard deviation. Staining scores were compared statistically in both groups. Shapiro-Wilk Test was used for the assessment of normality. The differences between the expressions levels of GSTO1, GSTP1, CYP1A1 in both groups were analyzed by Mann-Whitney U-test. The relationship between the expressions of GSTO1, GSTP1, CYP1A1 and clinical data were determined by Mann-Whitney U as well. Clinical data consisted of gender, smoking and drinking habits, reoperation, diseases including diabetes mellitus (DM), hypertension (HT), and atherosclerosis, and the use of antihypertensive and anticoagulant drugs. The Kruskal-Wallis test was used for comparing non-normal distributed data among groups. The relationship between the expression of GSTO1, GSTP1, and CYP1A1, and the ages of the study and control group patients were searched with Spearman's Rank Correlation Test. A statistically significant means a p value of less than 0.05.

Results

The current study included 43 subjects (29 in study group, 14 control). The mean age was 54 years (range, 17–76) in the study group and 56 years (range, 12–76) in the control category.

In the study and the control groups, the protein expression of CYP1A1 isozyme was seen at the rates of 27.6% and 50%, respectively. GSTP1 isozyme protein expression at 20.7% was observed in the study group while no expression could be detected in the series of control. No one in the category of controls demonstrated GSTO1 protein expression and one subject in study (3.4%), see (Figure 1).

The results indicated with 95% certainty that the data does not adapt the normal distribution according to the Shapiro-Wilk test. The differences between the levels of GSTO1, GSTP1, and CYP1A1 in both groups were investigated by Mann-Whitney U Test. The GSTO1, GSTP1, and CYP1A1 protein expression levels in both groups were shown in Table 1. Statistically significant differences in the levels of GSTO1 and CYP1A1 protein expression was not observed between the study and the control groups ($p > 0.05$). The increases in the expressions of GSTP1 and GSTO1 isozymes were observed to be positively correlated with the ratio of 38.3% (Spearman's correlation, $p < 0.05$).

Spearman's rank correlation analysis was conducted to reveal the relationship amid the ages of the patients and the levels of GSTO1, GSTP1, and CYP1A1 protein

Figure 1: Representative IHC analysis of GSTO1, GSTP1 and CYP1A1 expressions in tissues. IHC, Immunohistochemistry.

expression. A correlation was found between the expression of CYP1A1 and the age of the patients ($p < 0.05$, 30.5%). However, there was no statistically significant association between the age and the expression of GSTO1 and GSTP1 ($p > 0.05$). There was no significant correlation between the expressions of proteins and the following: sex, antihypertensive and anticoagulant drug usage, diseases such as diabetes mellitus, arterial hypertension, acute rheumatic fever, and atherosclerosis; smoking and drinking habits, and reoperation status ($p > 0.05$). The Kruskal-Wallis Test result suggested that statistically significant difference was not detected between MVI degree and expression levels of GSTO1, GSTP1, and CYP1A1 ($p > 0.05$).

Protein expressions of CYP1A1, GSTO1 and GSTP1 isozymes in the classes of control and study were shown in Table 1 and figured in Figure 2.

Discussion

In this article, we searched the immunohistochemical expression characteristics of GSTP1, GSTO1, and CYP1A1 in patients with rheumatic and ischemic mitral valve disease. The findings indicated that the expression of GSTP1 isoenzyme was higher in the rheumatic than that in the ischemic mitral valve disease patients ($p < 0.05$).

Many potential causes of rheumatic mitral valve disease have been reported, but there is still no consensus on the etiopathogenesis of rheumatic mitral valve disease. It has been proposed that oxidative stress and its subsystems are found in the reason of rheumatic mitral valve disease [11, 12]. There is delicate balance between reactive oxygen species and detoxification mechanisms

and oxidative stress is an unbalanced condition. This has an important role in the rheumatic mitral valve disease development. Oxidative stress has damaging effects on cells and tissues through cytotoxic effect and oxidative damage mechanisms [13, 14].

Some procarcinogens necessitate biotransformation and metabolic activity for binding to Deoxyribo Nucleic Acid (DNA); phase I enzymes supply this metabolic activity [15]. CYP enzymes catalyze the transformation of procarcinogens into genotoxic electrophilic products [16].

GST enzymes are classified into 8 groups [17]. GST has a function in antioxidation by catalyzing a reaction that neutralizes the electrophilic products of Phase I reactions [18]. GSTs such as GSTM1 (μ), GSTT1 (θ) and GSTP1 are potentially responsible for the detoxification of carcinogenic compounds and also play an important role in cellular protection by binding glutathione to reactive electrophiles [19].

In our study, we examined GSTP1, GSTO1 and CYP1A1 subtypes by immunohistochemical staining. In our examination of the ischemic mitral valve insufficiency in the control group patients by immunohistochemical staining; GSTP1, GSTO1, and CYP1A1 were expressed (Table 1). GSTP1 expression was higher in the rheumatic mitral valve disease tissues. GSTO1 and CYP1A1 were expressed at similar levels in control and rheumatic mitral valve disease tissues.

GSTs make several xenobiotics harmless. GSTs take care of tissues from injurious effects of oxidative stress convinced by chemical tissue damage.

The protein expressions of GSTP1, GSTO1, and CYP1A1 in rheumatic mitral valve disease tissue did not searched before according to the current literature. Immunohistochemical staining of GSTs and CYPs isoenzymes declare increased GSTP1 expression in the study group related to the class of control. We did not detected significant diversity in GSTO1 and CYP1A1.

After the reaction of the CYP P450 family with drugs, oxidative products are released. [20, 21]. The CYP P450 enzyme family is found at the bottom of several reactions of living beings [22]. CYP1A1, CYP1B1, CYP2E1, CYP2B6, and CYP3A are the five most dominant members of the CYP P450 family [23]. CYP1A1 and CYP1B1 are related to carcinogenesis due to their role on the metabolism of polycyclic aromatic hydrocarbons [24].

In one article, expression of similar enzymes was investigated in Ascending Aortic aneurysm; consequently expressions were detected significantly higher in that patients in accordance to those who had normal aortic tissue ($p < 0.05$) [25].

Table 1: Demographic data and GSTO1, GSTP1, CYP1A1 levels in the study and the control groups.

	Study group (n = 29)	Control group (n = 14)	p-Value
Age, mean (range)	54 (17–76)	56 (12–76)	0.675
Sex (F/M)	17/12	5/9	0.167
BSA (m ²)	1.80 ± 0.17 ^b	1.81 ± 0.16 ^b	0.816
Labored respiration	29/29 ^a (100%)	14/14 ^a (100%)	
EF (%)	55.21 ± 6.71 ^b	51.93 ± 10.58 ^b	0.236
MR degree	0: 1 1: 4 2: 10 3: 13 4: 1	0: – 1: – 2: – 3: 6 4: 8	0.000
SPAP (mmHg)	51.03 ± 12.06 ^b	45.28 ± 8.82 ^b	0.128
Mitral calcification	3/29 ^a (10.3%)	0/14 ^a	0.714
DM	4/29 ^a (13.8%)	5/14 ^a (35.7%)	0.102
Arterial Hypertension	9/29 ^a (31%)	6/14 ^a (42.9%)	0.458
Acute Rheumatic Fever	4/29 ^a (13.8%)	0/14 ^a	0.152
Atherosclerosis	7/29 ^a (24.1%)	14/14 ^a (100%)	0.000
Barlow disease	0/29 ^a	1/14 ^a (7.1%)	0.152
Infective endocarditis	0/29 ^a	0/14 ^a	1.00
Abnormal liver function	0/29 ^a	0/14 ^a	1.00
Infection	0/29 ^a	0/14 ^a	1.00
Smoking	10/29 ^a (34.5%)	3/14 ^a (21.4%)	0.395
Alcohol use	3/29 ^a (10.3%)	2/14 ^a (14.3%)	0.714
Drugs			
Beta blocker	12/29 ^a (41.4%)	8/14 ^a (57.1%)	0.343
ACE inhibitor	7/29 ^a (24.1%)	8/14 ^a (57.1%)	0.034
Ca ⁺⁺ channel blocker	11/29 ^a (37.9%)	2/14 ^a (14.3%)	0.119
Acetylsalicylic acid	4/29 ^a (13.8%)	5/14 ^a (35.7%)	0.102
Clopidogrel	1/29 ^a (3.4%)	1/14 ^a (7.1%)	0.600
Coumadin	9/29 ^a (31%)	1/14 ^a (7.1%)	0.019
Reoperation	3/29 ^a (10.3%)	0/14 ^a	0.152
GSTO1			0.494
Negative	28/29 (96.6%)	14/14 (100%)	
Positive	1/29 (3.4%)	0/14 (0%)	
GSTP1			0.0456
Negative	23/29 (79.3%)	14/14 (100%)	
Positive	6/29 (20.7%)	0/14 (0%)	
CYP1A1			0.156
Negative	21/29 (72.4%)	7/14 (50%)	
Positive	8/29 (27.6%)	7/14 (50%)	

ACE, Angiotensin converting enzyme; BSA, body surface area; DM, diabetes mellitus; EF, ejection fraction; MR, mitral regurgitation; SPAP, systolic pulmonary artery pressure. $p < 0.05$: statistically significant. ^aThe ratio of the number of positive patients to the total patient numbers (%), ^bmean ± standard deviation (SD).

In another study, plasma and tissue oxidative stress indices were found to be equal in various heart valve pathologies [11].

Plasma levels of advanced oxidation protein products and highly sensitive C-reactive protein levels were investigated and found significantly higher in rheumatic valve pathology [26].

In this research, the expression of GSTP1, GSTO1, and CYP1A1 isoenzymes did not show any association with

demographic variables. A wide range of researches may report new findings about valvular pathogenesis.

Eventually, GSTP1 expression increase in rheumatic mitral valve disease may be related to increased activation of GST for detoxification mechanism in rheumatic mitral valve disease. Also CYP1A1 and GSTO1 expressions are important enzymatic systems in rheumatic mitral valve disease patients. These findings suggest that rheumatic mitral valve disease is an oxidative stress

Figure 2: Immunohistochemical staining of GST and CYP isoenzymes in endocardial cells (Arrows in two figures point to endocardial epithelium). (A) positive staining of CYP1A1(40X), (B) CYP1A1 negative expression (40X), (C) GSTO1 negative expression (40X), (D) GSTP1 positive expression (40X), (E) GSTP1 negative expression (40X).

condition. Further investigations are warranted for better clarification.

According to our outcomes, we think that rheumatic mitral valve disease is unbalanced oxidative mechanism. Both of oxidative and antioxidative systems were found to increased in the rheumatic mitral valve disease. In pathology of rheumatic valvular disease, we detected GSTO1 and CYP1A1 expressions are not so meaningfull.

Within the scope of our study, GSTP1 enzyme is crucial function antioxidant defines method by prepare fusion counterproductives with GSH at rheumatic mitral valve tissue, and GSTP1 isozyme that is a member of this enzyme family was found to be increased. GSTP1 quantities were substantial amount greater in rheumatic mitral valve tissue than GSTO1 quantities indicates GSTP1 can be lay on the basis of this pathology. We know that, there

is not any study in English literature on GST levels heart valve of patients with rheumatic mitral valve disease.

Conclusion

Harmful effects removal feature of GSTP1 enzyme was established distinct in rheumatic mitral valve cases contrast the normal valve tissue group. In rheumatic mitral valve disease cases, other enzymes that reduce the harmful effects of free radicals affected by the GST family of enzymes should also be examined. Increased GSTP1 expression and the effect of GSTP1 isozyme structural causes of inflammatory mitral tissue have shown in this histologically designed study.

Nevertheless, knowing that oxidation enzymes are involved in rheumatic valvular heart diseases is exciting for future studies.

Study limitations

The control group was very meticulously chosen for mitral valve unaffected by rheumatism. For this reason, mitral tissue samples in the ischemic valve disease group was lower.

Since rheumatic heart valve diseases are present in Turkey, there were no problems in terms of rheumatic valve tissue in the rheumatic group. Due to limited budget and immunohistochemical staining kits, more patients could not be included in the study.

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