

Expression of the CD44 Protein in the Heart Valves Affected with Rheumatic Heart Disease

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Abstract: Problem statement: The primary aim was to study the expression of CD44 protein in the heart valves removed surgically for either stenosis or regurgitation and to study the morphology of valves using histochemical staining. **Approach:** We studied 107 valves which were collected from the International Centre for Cardio Thoracic and Vascular Diseases. **Results:** Aortic and mitral valves were obtained from the centre and processed in research pathology lab. CD44 protein is a receptor for the ligand hyaluronic acid which causes inflammation in the heart valves. A total of 107 valves were studied. Of the 61 mitral valves studied 38 (62.2%) showed CD44 positivity as brownish granules within the cytoplasm. Of the 46 aortic valves studied 19 (41.3%) showed CD44 positivity as brownish granules within the cytoplasm. The valves showed evidence of past inflammation showing thick and thin walled blood vessels and lymphocytes. CD44 protein increased in 62.2% of mitral valves, of which 60.5% were from patients presenting when they were less than 40 years old. CD44 protein increased in 41.3% of aortic valves, of which 63.15% were from patients presenting when they were less than 40 years old. CD44 positivity was seen in 57 valves as brownish granules within the cytoplasm of the cell. CD44 protein increased in 53.27% of mitral and aortic valves, of which 63.15% were from the males patients. CD44 protein increased in 53.27% of mitral and aortic valves, of which 36.84% were from the females patients. **Conclusion/Recommendations:** The results showed CD44 is over expressed in the heart valves removed surgically for stenosis or regurgitation where the hyaluronic acid content is high.

Key words: Hyaluronic acid, rheumatic heart disease, histochemical staining, vascular diseases, mitral valves, aortic valves

INTRODUCTION

Rheumatic fever and its cardiac sequelae continue to be an enigma as to why some people are predisposed to cardiac involvement while others are not. That is due in predisposed patients to an autoimmune reaction to untreated or partially treated group A streptococcal pharyngitis has been established. The component of the bacteria responsible for the antigen cross reactivity to the heart tissue has not been established. Cross reactivity was initially observed in extracts of the streptococcal cell wall. The M protein has been the most extensively studied component of the streptococcus (Fischetti, 1991). The streptococcal cell wall has a high concentration of N-acetyl-glucosamine, which is also found in high concentrations in valvar tissue (Veasy and Hill, 1997).

The group a streptococcus capsule is composed of hyaluronic acid, which is also increased in valves (Marcon *et al.*, 1988). CD44 is a cell surface receptor for several extracellular matrix components including hyaluronic acid and implicated in tumor cell invasion and metastasis (Okamoto *et al.*, 1999). As CD44 protein is a common component we hypothesized that this may be responsible for the cross reactivity.

MATERIALS AND METHODS

We studied 107 mitral and aortic valves removed surgically for either stenosis or regurgitation. The valves were examined grossly for commissural fusion; thickening of leaflets; shortening, thickening and fusion of chordae tendinae and presence of these features was taken as evidence of infection.

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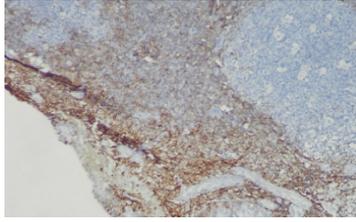


Fig. 1: Control (Tonsil tissue)

After gross examination, a sample of the whole length of the leaflet was taken from the leaflet avoiding calcified areas and was subjected to routine histopathological processing. After gross examination, a sample of the whole length of the leaflet was subjected to routine histopathological processing and Immunohistochemical staining using anti-CD44 (phagocytic glycoprotein-1) antibody and tonsil tissue was used as a control (Fig. 1). The morphology of the heart valves was studied by staining the sections with hematoxylin and eosin, elastic van Gieson (where collagen is stained reddish brown and elastic fibers are stained black) and Alcian blue at pH 2.5 highlighting the hyaluronic content of the valves (stained bluish green). The sections were examined under 4×10 and 40×10 magnifications. The CD44 protein was expressed as brownish granules inside the cytoplasm of the cell. Microscopic evidence of over expression of the CD44 protein was noted.

RESULTS

A total of 107 valves were studied (Table 1 and 2). Of the 61 mitral valves studied 38 (62.2%) showed CD44 positivity as brownish granules within the cytoplasm (Fig. 2). Of the 46 aortic valves studied 19 (41.3%) showed CD44 positivity as brownish granules within the cytoplasm (Fig. 3). The valves showed evidence of past inflammation showing thick and thin walled blood vessels and lymphocytes. CD44 protein increased in 62.2% of mitral valves, of which 60.5% were from patients presenting when they were less than 40 years old. CD44 protein increased in 41.3% of aortic valves, of which 63.15% were from patients presenting when they were less than 40 years old. CD44 positivity was seen in 57 valves as brownish granules within the cytoplasm of the cell. CD44 protein increased in 53.27% of mitral and aortic valves, of which 63.15% were from the males patients (Table 3). CD44 protein increased in 53.27% of mitral and aortic valves, of which 36.84% were from the females patients (Table 3). The morphology of the heart valves was also studied using routine histochemical staining methods.

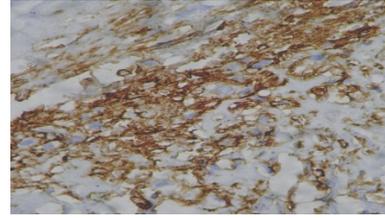


Fig. 2: CD44 expression in mitral valve (S 276M (40 X) mitral valve, CD44 is expressed in the infective endocarditis sample, Male, 57 years old)

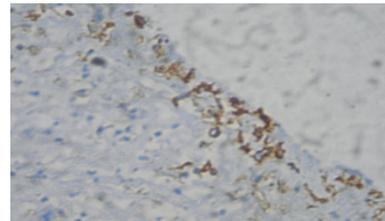


Fig. 3: CD44 Expression in Aortic valve S 51A (40 X) Aortic valve, CD44 is expressed in the infective endocarditis sample, Male, 30 years old

Table 1: Distribution of the valves with the CD44 expression

Total number of valves	Mitral valve (n = 61) No. positive	Aortic valve (n = 46) No. positive
107	38 (62.2%)	19 (41.3%)

Table 2: Distribution of the valves by the patients' age with and without the CD44 expression

CD44 expression	Mitral valve (n = 61)		Aortic valve (n = 46)	
	No. negative	No. positive	No. negative	No. positive
Age at operation (years):				
<30	2	14	1	8
31-40	5	9	4	4
41-50	9	8	13	6
<50	7	7	9	1

Table 3: Distribution of the valves by patients' sex with the CD44 expression

Total number of valves showing CD44 positivity	Males (n = 61) No. positive	Females (n = 46) No. positive
57	36 (63.15%)	21(36.84%)

DISCUSSION

We observed that a significant number of valves removed for either incompetence or stenosis secondary to rheumatic heart disease had an increased expression of CD44 protein. Here, a significant number of patients with increased expression of CD44 protein presented in the fourth decade with rheumatic involvement

suggesting that this component may predispose valves to rheumatic heart disease. This is especially evident in both the aortic and the mitral valves. CD44 protein increased in 62.2% of mitral valves, of which 60.5% were from patients presenting when they were less than 40 years old. CD44 protein increased in 41.3% of aortic valves, of which 63.15% were from patients presenting when they were less than 40 years old.

Hyaluronic acid is a high molecular weight linear polysaccharide composed of repeating sequences of glucuronic acid and N-acetyl-glucosamine and CD44 acts as a receptor for colonization of the pharynx by group A streptococcus (Cywes *et al.*, 2000). Positivity for CD44 was seen in the valvar tissue in 57 out of 107 cases. Since, some of the heart valves have high hyaluronic acid content CD44 is over expressed in these valves.

CONCLUSION

Our study shows that there is an increased expression of CD44 protein in the heart valves. Further experiments with sheep antibodies raised against purified CD44 protein and cross reacting with streptococcal capsule and valvar tissue are underway.

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