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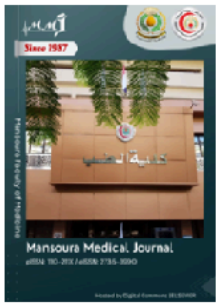

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DETECTION OF CHLAMYDIA PNEUMONIAE IN DISEASED AORTIC AND MITRAL VALVES DIAGNOSTIC DILEMMA

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ABSTRACT

Chlamydia Pneumoniae "CP" is a common pathogen that has been linked to coronary artery disease . Also , CP antigen has been demonstrated in valve biopsy specimens from patients with acquired aortic valve stenosis & in patients with culture negative endocarditis . The aim of this paper is to study the presence of CP in both aortic & mitral valves by using polymerase chain reaction "PCR" & its relation to the pathology of the valve . 27 patients "16 males" who underwent aortic & mitral valve replacement were studied . Key demographic & clinical data were collected including age , gender , past history of rheumatic fever , NYHA class , preoperative 12 leads ECG , Chest X-ray & Echocardiography . One portion of the valve leaflet was sent for pathologic examination to detect the nature of valve disease e.g. rheumatic or non-rheumatic & the other portion was sent for PCR study . The age ranged from 19 to 55 years with mean valve 36.48 ± 11.46 . 5 patients had isolated aortic valve stenosis , 12 had mitral valve disease & 10 had double valve disease . Aschoff body & cells were seem in 11 patients and 5 patients had non-rheumatic degenerative aortic stenosis . 7 out of 27 cases (25.9%) were PCR positive , 5 of them were isolated non-rheumatic aortic stenosis and represent (33.3%) of aortic cases . The remaining 2 PCR positive cases were rheumatic mitral valve disease one of them was se-

vere isolated mitral regurgitation due to native valve endocarditis . CP infection was common with non-rheumatic aortic valves "P=0.045" & also with stenotic lesions than regurgitant ones "P=0.045" . Culture negative endocarditis maybe due to CP infection & also CP maybe present in rheumatic mitral valve most probably as passengers .

INTRODUCTION

Chlamydia pneumoniae (CP) is a common respiratory pathogen that has been linked to coronary artery disease (CAD) and has been demonstrated in specimens from atherosclerotic lesions [1-4]. All Chlamydia species have been documented to cause heart infections. In 1974, Ward and Ward demonstrated the presence of Chlamydia antigen in atrial and valve biopsy specimens from patients with acquired aortic valve stenosis [5]. CP has also been suspected of being the etiologic agent in cases of bacterial culture-negative endocarditis and an association with myocarditis has been also documented [6-8].

This paper is an attempt to study the presence of CP in excised aortic and mitral valves by using polymerase chain reaction (PCR) as will as the relation between CP & Pathology of the valve (rheumatic & non-rheumatic) .

PATIENTS AND METHODS

27 patients (16 males) who are subjected to aortic and/or mitral valve replacement in cardiothoracic surgery department, Mansoura Faculty of Medicine, were studied. At the time of hospitalization, key demographic and clinical characteristics were collected including age, gender, past history of rheumatic fever, NYHA Class, Preoperative 12-leads ECG, chest x-ray and echocardiography were done for each patient.

Blood tests for rheumatic fever were done e.g. C. reactive protein and antistreptolysin-O-titre (ASO) .

Two-dimensional (2D), M- mode and color Doppler transthoracic echocardiographic studies were performed in all patients (Acuson, model 128XP, 2.5/3.5-MHz probe). Two-dimensional parasternal long-axis, short-axis and apical four-chamber views were recorded to visualize the anterior and posterior mitral leaflets adequately. M-mode echocardiography was performed according to the recommendations of the American

Society of Echocardiography . Mitral valve area was also measured by the pressure half-time method. Color Doppler was used to detect the presence of MR, and MR severity was assessed by a semiquantitative method. We measured the percent ratio of the maximal flow disturbance produced by the MR jet to the left atrial area and graded it according to the Nagle criteria (angiographic) as follows: 1) <20% was considered mild; 2) 20-40% was graded as moderate; and 3) >40% was considered severe. Mitral valve prolapse (MVP) was diagnosed when the apposed mitral leaflets were displaced posterior to the annulus line ≥ 2 mm in late systolic or ≥ 3mm for holosystolic MVP on M-mode echocardiography. Holosystolic M-mode MVP was accepted only if confirmed by leaflet billowing into the left atrium on the parasternal or apical long-axis views .Rheumatic mital valve disease was diagnosed when the mitral leaflets were thickened and calcified and had evidence of fusion of mitral commissures or subvalvular appa-ratus on 2D examination (24,25).

Two-dimensional assessment of the aortic valve was performed from the parasternal long- and short-axis and apical long-axis views. A diagnosis of degenerative valvular disease was made on the basis of clinical criteria (no history of rheumatic disease) and the echocardiographic finding of thickening and increased echogenicity of the cusps (excluding the free edges) with reduced systolic opening. The degree of calcification of the aortic valve was assessed on the basis of recently proposed echocardiographic criteria .Each valve leaflet was graded on a scale of 0 (normal) to 3+ (severe) for leaflet thickening and calcification. A total valve score was calculated as the sum of the individual leaflet scores divided by the number of leaflets. This score was used to classify the aortic valve as normal or abnormal (score ≥0.25). The confirmation of calcification of the valvular leaflets of the aortic valve at surgery was an additional criterion. Aortic jet velocity (m/s) and aortic valve area (cm^2) were measured at echocardiography and taken as parameters of aortic stenosis severity. Aortic stenosis was defined as thickened leaflets with reduced systolic opening on 2D imaging and an increased anterograde velocity (≥2.5 m/s by continuous wave Doppler ultrasound) across the valve. Patients with echocardiographic findings suggestive of congenital valve stenosis

(identification of two cusps in systole and systolic cusp doming, highly asymmetric thickening or both) or rheumatic valve stenosis (commissural fusion and mitral valve involvement) were also included (26).

Intraoperative assessment of the valves was done by the surgeon during operation for evidence of fibrosis, calcification or vegetation. Management of valve leaflets was as follow:

One leaflet was immersed in 10% formaline solution for pathologic examination to detect the nature of valvular disease e.g. rheumatic or non-rheumatic nature.

Another portion of the leaflet was immersed in normal saline for PCR study.

The excised valves were preserved in neutral buffered formalin and sent for pathology. 4 mu thick Paraffin sections were stained with haematoxylin & eosin and were examined for the presence of Aschoff bodies, fibrosis, calcification and any other pathological findings. Masson trichrome stain was performed for assessment of fibrosis. Presence of macrophages, fibrinoid necrosis, and the large caterpillar- like cells were searched for to diagnose rheumatic activity. PAS stain and Giemsa stains were applied as routine work.

Bacteriology :

DNA extraction: Frozen valve tissue in Chlamydia transport medium was divided in two parts, one for microbiologic analysis by conventional aerobic and anaerobic bacterial culture, and the other for PCR.

For PCR: the frozen tissue were homogenized in a DNA extraction buffer containing 10 mM Tris, pH 8.0, 100 mM EDTA, 0.5% SDS, and 20 ug/ml pancreatic Rnase. Deoxyribonucleic acid (DNA) was purified according to the Holland et al., 1990 (27) , and resuspended in 100uL of 10 mM Tris, pH 8.0, and 1 mM EDTA.

PCR assay: The primers HL-1 and HR-1 will amplified a 437-bp C. pneumoniae-specific DNA sequence .

Each reaction mixture contained in a total reaction volume of 100 L with 5mM MgCl2, 10 mM Tris-HCl, pH 8.3,5 mM KCl, gelatin 0.01% w/v, Triton X100 0.1% v/v, 200 µM of deoxyribonucleotide, 50 pM each of primers HL-1(GTT GTT CA TGA AGG CCT ACT) and HR-1(TGC ATA ACC TAC

GGT GTG TT(, and Taq polymerase (Boehringer Mannheim) 4U. Amplification was done for 40 cycles, each cycle consisted of the following: denaturation at 94 C for 1 min, annealing at 55 C for 1 min, and primer extension at 72 C for 1min.

Amplification products were fractionated on 2% agarose gel containning ethidium bromide (0.5ug/ml) and examined under UV light using the transilluminator FBTIV-88 (from Fisher scientific Pittsburg USA) and gels containing bands were pictured using the built-in-Polaroid camera (Photo-Documentation, Hood FB-PDH-1216 from Fisher scientific Pittsburg USA). Positive specimen will produce a 474bp DNA fragment on ethidium bromide-stained agarose gels (28&29).

RESULTS

27 patients (16 males) were studied, the age ranged from 19 to 55 years with mean value 36.48 ±11.64. According to NYHA functional classification, 4 were in class II, 15 were in class III and 8 were in class IV. 15 patients had aortic valve disease and 12 had mitral valve disease. Cardiothoracic ratio ranged from 50 to 70% with mean value 59.92 ± 5.33. Our Results were shown in tables 1 to 3 and figures 1 to 3 .

Echocardiography : 15 patients had aortic valve disease: 5 with isolated severe aortic stenosis without mitral valve affection, the remaining 10 cases were associated with mitral valve disease, 6 of them had mild aortic stenosis and 4 had moderate aortic stenosis. Aortic regurgitation was absent in 5 cases, while the remaining 10 cases had various degrees of aortic regurgitation (mild in one case, moderate in 6 cases and severe in 3 cases).

12 patients had isolated mitral valve disease: Mitral stenosis was mild in 3 cases, moderate in 7 and severe in 2 cases. Mitral regurgitation was mild in 1 case, moderate in 1 case and severe in 10 cases.

Calcification was present in 12 cases (7 aortic and 5 mitral). Vegetation was present in one patient with severe mitral regurgitation.

Intraoperative assessment : During valve replacement, the surgeon had assessed the valves macroscopically. Various degrees of fibrosis were seen in all patients. Calcification was seen in 12 (44.4%) cases (7 aortic

and 5 mitral). Large vegetation was seen in one case with native mitral valve endocarditis.

Pathology : Aschoff body and Aschoff cells were seen in 11 patients (5 aortic and 6 mitral) i.e. rheumatic activity was present in 11 cases. Fibrosis was present in 19 cases (9 aortic and10 mitral), calcification was present in 12 cases (7 aortic and 5 mitral), and chronic nonspecific inflammation was present in 15 cases (10 aortic and 5 mitral).

We have 5 cases with non-rheumatic degenerative aortic stenosis. They have negative history for rheumatic fever, not associated with mitral valve affection and pathologic examination showed no Aschoff body or cells. They have fibrosis and chronic nonspecific inflammation and they were negative for C. reactive protein and ASO titre .

Bacteriology : As shown in table 3; 5 aortic cases out of 15 (33.3%) were PCR positive for CP. CP infection was common with non-rheumatic aortic valves and this relation was statistically significant, (P= 0.045). CP infection was significantly prevalent in stenotic lesions than regurgitant lesions, (P= 0.045). These correlations confirmed the results of others (3, 12).

In our series we had two mitral cases that were PCR positive for CP. One of them was rheumatic double mitral valve disease and the other was severe isolated mitral regurgitation due to native valve endocarditis.

Table 1: Age & Sex Distribution of the Patient group

Age Group	Sex		No. of Patients	Percentage
	Male	Female		
11-20 Years	2	2	4	14.9
21-30 Years	8	5	13	48.0
31-40 Years	1	2	3	11.1
41-50 Years	3	1	4	14.9
51-60 Years	2	1	3	11.1
Total	16	11	27	100 %

The age of patients ranged between 19&55 years with mean age 36.48 ±11.64 .

Table 2 : Diagnosis of Rheumatic & non-rheumatic Patients

	Rheumatic (22 Patients)	Non-rheumatic (5 Patients)
Valve Lesion	DVD 10/22 (45%) MVD 12/22 (54%)	AS 5/5 (100%)
Past history of Rheumatic fever	12/22 (54%)	0/5 (0%)
Aschoff nodule	11/22 (50%)	0/5 (0%)
Positive ASO titre	12/22 (54%)	0/5 (0%)
Positive C. reactive Protein	11/22 (50%)	0/5 (0%)

DVD = Double valve disease .
MVD = Mitral valve disease .
AS = Aortic Stenosis .

Table 3 : Chlamydia pneumoniae in excised aortic valves .

Category of AVD	CP +	CP -	P value
Rheumatic	4/10 (40%)	6/10 (60%)	NS
Non-rheumatic	4/5 (80%)	1/5 (20%)	0.045
Isolated stenosis	4/5 (80%)	1/5 (20%)	0.045
Stenosis combined with regurgitation	4/10 (40%)	6/10 (60%)	NS

Chlamydia Pneumoniae is common in non-rheumatic (P=0.045) & Isolated aortic stenotic patients (P=0.045)

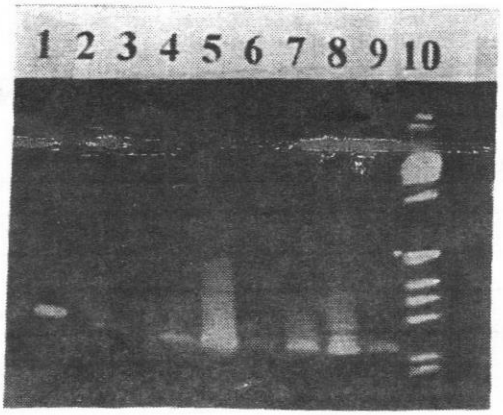

Figure 1 : A representing gel stained with ethidium bromide containing the amplified PCR products opposite to 474bp for C. pneumoniae DNA . Lane(10) shows HaeIII-digested phagex174 molecular size marker. Lane (1,2,4-9) show the PCR products of some positive cases. Lane 3 shows negative cases.

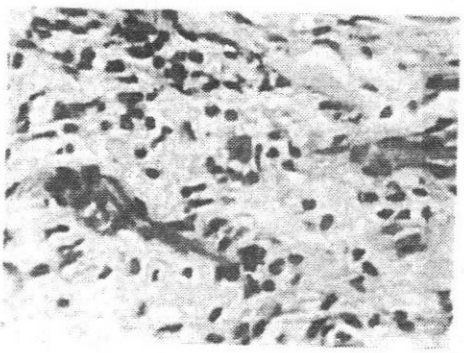

Figure 2 : Rheumatic endocarditis: Aschoff body, perivascular macrophages with large caterpillar- like cell. (H&Ex400).

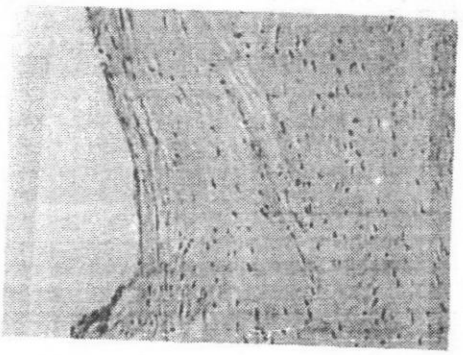

Figure 3 : Non-rheumatic endocarditis. There is some fibrosis, but lacking significant inflammatory cellular infiltrate (H&Ex400).

DISCUSSION

Chlamydia pneumoniae (CP) has been found previously in atherosclerotic lesions [9,10,11,30,33]. Also Chlamydia antigen has been previously shown to be present in acquired valvular disease [5].

Our series (27 patients) was larger than the series of Juvonen et al. [12] who studied 17 patients. In addition, we studied both aortic (15 cases) and mitral (12 cases) valves but Juvonen et al. [12] studied only aortic valves.

In our study the incidence of CP in aortic valves (33.3%) is slightly lower than that in the series of Juvonen et al.[12] who found CP in 9 (53 %) out of 17 aortic patients. Our CP-positive aortic cases were non-rheumatic because they had isolated aortic stenosis without mitral valve affection, they did not give past history of rheumatic fever and pathologic examination showed chronic nonspecific inflammation with calcific degeneration. Also they were negative for ASO titre and C. reactive protein .

Chlamydia can damage heart tissues causing acute and subacute valvular and other heart infections [13&31] and persistence is well known feature of Chlamydia infections. The pathogenesis of persistent CP infection may bear some similarities to that of trachoma. Up to 90% of the population of some developing countries are infected with C. trachomatis, but its manifestations depend on the patients immunologic response, so that only a small proportion develop trachoma, the majority being nonsymptomtic or having a mild eye infection [14]. It is possible that valvular inflammations associated with CP may vary from rare acute and subacute endocarditis to chronic valvular inflammation in immunologically susceptible persons. Lipopolysaccharides of CP can provoke and/or contribute to the endothelial injury, feed the inflammation and stimulate the production of inflammatory cytokines [15].

We did not find in the literature rheumatic mitral valve disease with CP infection but we have 2 rheumatic mitral cases with this finding. We think that, although our results showed the presence of CP in diseased mitral valve but it does not establish the etiologic relation between the two, it is possible, of course, that CP infections may only be passengers that lodge in diseased mitral valve tissues.

In our study, one of the 2 mitral cases with PCR positive for CP was native mitral valve endocarditis. This finding is different from the finding of marrie et al. [16] who found native valve endocarditis due to CP but in aortic position. Our case with Chlamydial endocarditis was female patient, 28 years old and had rheumatic severe mitral regurgitation admitted with clinical picture of endocarditis but blood cultures were negative and after mitral valve replacement PCR was positive for CP. We did not find in the literature a case with culture-negative mitral valve endocarditis due to CP infection.

Infection with Chlamydia species rarely results in endocarditis and when it does occur, it is blood culture-negative variety i.e. clinical evidence compatible with endocarditis and blood cultures were negative for both aerobes and anaerobes [17-20]. The published cases of Chlamydial endocarditis are 8 cases, C. psittaci caused 6 cases, C. trachomatis one case and CP one case. The clinical course may be acute, subacute or indolent [17]. The TWAR strain of Chlamydia is named for the laboratory code of the first two isolates (TW-183 and AR-39), this organism has been implicated in both upper and lower respiratory tract infections, so the name Chlamydia pneumoniae has been given to this new species and it has been shown to be distinct from C. trachomatis and C. psittaci [21-23]. We and others [16] have limited experience with the therapy of Chlamydial endocarditis to make any recommendations. CP is susceptible to tetracycline and erythromycin but resistant to penicillin, ampicillin and sulfsuxazole [24&32].

CONCLUSION

(1) CP is frequently present in non-rheumatic degenerative aortic valve stenosis and may play an etiologic role in the development of this disease.

(2) CP may be present in rheumatic mitral valve most probably as passengers and we can not establish the etiologic relation between the two because we have only one case in our series, which is not enough at all to make recommendation.

(3) Culture-negative endocarditis may be due to CP but we can not recommend certain drug regimen for this condition and further studies will be needed to through lights on this subject.

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