Histomorphological Patterns in Myocarditis in Postmortem Specimens- A Descriptive Observational Study over a Period of 4 Years at a Tertiary Care Centre in Kerala

Cicy Petta Joseph[1], Lekshmi Devi P.[2], Letha V.[3], S. Sankar[4]

[1]Department of Pathology, Government Medical College, Kottayam, Kerala, India. [2]Department of Pathology, Government Medical College, Kottayam, Kerala, India. [3]Department of Pathology, Government Medical College, Kottayam, Kerala, India. [4]Department of Pathology, Government Medical College, Kottayam, Kerala, India.

ABSTRACT

BACKGROUND

In cases of sudden death, myocarditis is not an unusual finding especially when communicable diseases of viral origin are on the rise. Detection of myocarditis poises a diagnostic challenge to clinicians due to the absence of specific symptoms and there is a possibility of this being masqueraded as myocardial infarction. Examination of autopsy specimens are of utmost importance to detect the underlying etiology. For diagnosis of myocarditis histopathological examination of cardiac tissue is the gold standard. This is carried out based on Dallas criteria.

Aim- This study was done to assess the, gross and histopathology findings in myocarditis in post mortem specimens in a tertiary care hospital in Kerala over a span of 4 years.

METHODS

This is a descriptive observational study. All cases of Myocarditis diagnosed in the Department of Pathology, Medical College, Kottayam during the period of 4 years from January 2012 to December 2015 were included. Age distribution, gross and histopathologic findings were studied using the registers, post mortem specimens and histopathology slides in the department. Immunohistochemical studies were done in paraffin blocks of all newly diagnosed cases of myocarditis and some previously diagnosed cases the data was analyzed using SPSS.

RESULTS

We received a total of 82 cases of myocarditis. The age distribution ranged from 75 days to 83 years. There was greater predilection for males accounting for 62% of cases. Inflammation was found in all cases and the predominant inflammatory cells were lymphocytes seen in 73% of cases. Myocyte necrosis was found in majority of the cases (98%).

CONCLUSIONS

Myocarditis was found to be an important cause of sudden death accounting for 0.15%.The age group affected was more in the 3rd and 4thdecade,which was comparable with other similar studies. Viral myocarditis constituted the majority of cases and mononuclear inflammation was seen in myocardium. Myocarditis was seen as part of systemic inflammation in 70% of cases. Application of Dallas criteria was helpful in diagnosis but correlation with clinical details and meticulous examination of coronaries are also needed to exclude conditions which may mimic myocarditis.

KEY WORDS

Myocarditis, Post Mortem, Viral.

Corresponding Author:
Dr. Lekshmi Devi P.,
Department of Pathology,
Government Medical College,
Kottayam, Kerala, India.
E-mail: elavumkal@gmail.com
lekshmydevi@yahoo.com
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BACKGROUND

Myocarditis is one of the important causes of sudden death especially in young adults. Diagnosis of myocarditis is often challenging in clinical practice due to the absence of specific symptoms and there is a possibility of this being masqueraded as myocardial infarction.[1] Though the underlying cause may be a viral infection in many cases, myocarditis is often idiopathic.[1]Any type of virus can affect the cardiac myocytes. There are several forms of Primary viral myocarditis which are defined by their clinical and pathological manifestations.[2] Viral myocarditis is one of the most common causes of myocarditis which ultimately leads to dialated cardiomyopathy in 30% of patients.[3]

For the diagnosis of myocarditis histopathological examination of cardiac tissue is the gold standard. This is carried out based on Dallas criteria.[1,2] Histopathological categorization for the diagnosis of myocarditis was provided in the Dallas criteria proposed in 1986.[2] This criteria requires an inflammatory infiltrate in the myocardium with associated myocyte necrosis or damage which should not be part of an ischemic event.[2] In suspicious cases, where there is no myocyte necrosis or >14mononuclear cells)/mm2 on light microscopy, perfoming immunohistochemical studies are helpful in arriving at a diagnosis. Many investigators have challenged this criteria in the past. Endomyocardial biopsy is an invasive procedure and is not possible in all suspected cases of myocarditis. Moreover there is chance of underdiagnosis since inflammation may be seen only in lateral wall of left ventricle in early cases of myocarditis where as, endomyocardial biopsy is done from the wall of right ventricle.[3] This might lead to false negative diagnosis due to sampling error. However in autopsy specimens a detailed sampling is possible. We have attempted to check the importance of Dallas criteria for diagnosis of myocarditis based on the histopathological examination of cardiac tissue of autopsy specimens we received in our department. This study was done to assess the, gross and histopathology findings in myocarditis in a tertiary care hospital in Kerala over a span of 4 years and an attempt to find the underlying etiology for those cases where it is possible. Immunohistochemistry (IHC) was done in borderline cases of myocarditis.

Objective

1. To analyse the histopathologic features of Myocarditis and categorise them in post-mortem cases.
2. To estimate the percentage of unexpected death due to myocarditis in postmortem cases.
3. To assess the usefulness of IHC in diagnosis of Myocarditis.

METHODS

Study Design

Descriptive observational study.

All suspected cases of Myocarditis diagnosed in the Department of Pathology, Government Medical College, Kottayam during the period of 4 years from January 2012 to December 2015 were analyzed. Age distribution and morphologic findings were studied using the registers, post mortem specimens, histopathology and IHC slides in our institution. IHC studies were done in paraffin blocks of all newly diagnosed cases of myocarditis and previously diagnosed cases. The study was accepted by the Institutional ethics committee (IRB 139/2017).

Study Tools

1. Instruments to take bits of tissues to be studied.
2. Reagents for tissue processing.
3. Instruments for making paraffin blocks and cutting thin sections from it.
4. Glass slides and cover slips for mounting.
5. microscope
6. Eosin- Haematoxyline staining.
7. Reagents for immunohistochemical studies.
8. Proforma to record serial number, Biopsy number, Name, age, sex, clinical investigations, gross, histopathology and immunohistochemical features.

Study Procedure

Clinical details provided along with each postmortem specimen of heart will be recorded first.Gross examination of the specimen will be done. All specimens will be fixed in formalin and embedded in paraffin. 4 microns thick sections will be stained with H & E for routine histological examination. Immunohistochemical staining was performed using mouse monoclonal antibody for CD3 and CD20.

Inclusion Criteria

All autopsy specimens of heart, entire heart or sections of heart with diagnosis of myocarditis received in the histopathology lab during the period of January 2012 to December 2015

Exclusion Criteria

Markedly autolysed specimens.

Statistical Analysis

The data was entered in Microsoft excel and further statistical analysis was done using Statistical Package for Social Sciences (SPSS) programme. Descriptive statistics was used and frequency tables were generated for age, sex, proportion of cases with coronary occlusion and various histopathological features like predominant inflammatory cell type, presence of myocyte necrosis and fibrosis.

RESULTS

We received a total of 82 cases of myocarditis over a period of four years. The year wise distribution is shown in figure 1. There was a sudden increase in the number of cases from the second year and the maximum number of cases, 44% occurred in 2013.

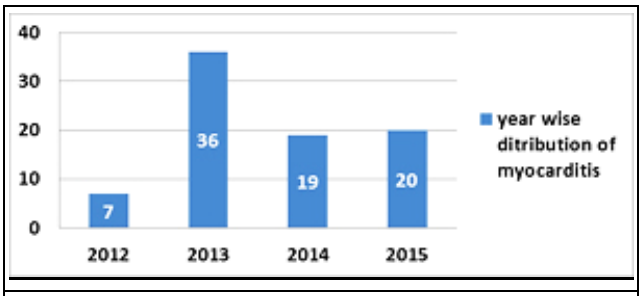

Figure 1. Year Wise distribution of Myocarditis

The age affected ranged from 75 days to 83 years, with a median age of 31 years. Males (51 cases) were more affected than females (31 cases) with a male to female ratio of 1.6:1.The maximum number of cases, 47.5% were seen to occur between the 3rd and 4th decade.

Decade wise distribution of myocarditis and relative frequencies of the different histopathologic types is shown in Table 1.

Age in Years	Viral Myocarditis (Mononuclear Cells)	Acute Myocarditis (Neutrophils)	C/c Myocarditis (Fibrosis)	Mixed (Mononuclear Cells & Neutrophils)	Total (82 Cases)
0 – 20	10	3	0	1	14
20 -40	26	4	0	9	39
40 -60	14	1	0	3	18
60-80	7	0	1	1	9
80-100	2	0	0	0	2
	59	8	1	14	

Table 1. Age distribution and the Relative Frequencies of different Histopathological Types of Myocarditis

Grossly majority of cases showed a flabby appearance. Patency of the coronaries were checked and it was found to be patent in 82.9% (68 cases) of the cases. The remaining 17.1% cases showed occlusion. However it was not severe enough to be fatal. The maximum percentage of occlusion detected was 60% which was in an 80 year old male who succumbed due to viral myocarditis.

The histopathological findings assessed in our study were presence of inflammation, necrosis and fibrosis. Figure 2 shows the proportion of cases with the predominant inflammatory cell type involved.

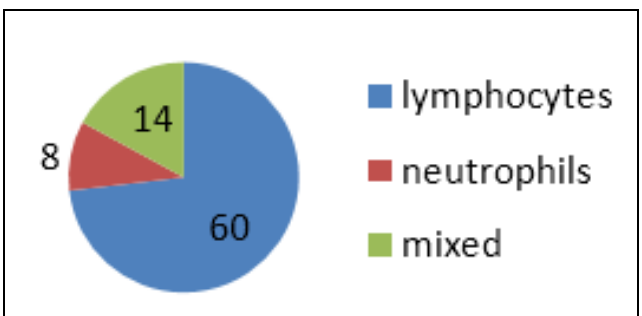

Figure 2. Relative Proportion of Myocarditis based on the Predominant Inflammatory Cell Involved

Myocyte necrosis was found in majority of the cases (98%). Neutrophilic infiltrate, myocyte necrosis and pericardial inflammation forming a pancarditis were also noted in some cases. Fibrosis of myocardium was seen in 1.2% cases only. In 70 %(57 cases) of cases myocarditis was seen to occur as a part of systemic involvement and all such cases showed pathologic findings in other systems also.23% of the cases showed involvement of heart only. The predominant organs affected were lungs, brain, kidneys. In 6% involvement of other systems could not be assessed.

IHC studies were done in 15 cases only and it showed nuclear positivity for CD3 marker in all of them.CD 20 was negative.

DISCUSSION

In developing countries like India, where the molecular diagnostic facilities are far from the reach of people, Dallas criteria still play an important role in diagnosis of myocarditis which present as sudden death. Based on Dallas criteria >14 mononuclear cells/mm ^{2} along with myocyte necrosis in myocardial tissue is considered as positive formyocarditis.[1]It is a diagnostic challenge, for the clinician due to its highly variable clinical presentation and also due to lack of definitive and specific features in most of the noninvasive investigations done routinely.[3]

Due to the heterogeneous nature of this disease, differential diagnosis of myocarditis is a great concern. Sometimes patients with right ventricular myocarditis can mimic arrhythmogenic right ventricular cardiomyopathy on the basis of clinical features and investigative finding regarding ventricular function. Myocarditis may also be indistinguishable from an ischemic heart disease since both may have chest pain, abnormal electrocardiogram and elevated enzyme biomarkers. Great care and stringent criteria must be applied while diagnosing suspected myocarditis. Because of this prevalence of myocarditis and this causing death is greatly underestimated, this can significantly be improved by analysing autopsy specimens of heart from sudden death cases where the exact cause is not evident.[4]

In our study we received a total of 82 cases. The predominant age group affected was between the 3rd and 4th decade with a median age of 31 years [Table 1].There was greater predilection for males accounting for 62% of cases (51 cases) andmale: female ratio was 1.6:1. This was comparable with studies of Passarino et al and ludden et al.[5,6]

Myocarditis can be induced by a number of different infectious agents and represents a significant cause of death especially in young individuals. Infectious causes of myocarditis are viruses, bacteria, and protozoa. Virus are the most important cause of myocarditis owing to the limited clinically evident symptoms and drastic outcome. Enteroviruses, (Especially Coxsackievirus) adenovirus, parvovirus B19, human herpesvirus 6, human cytomegalovirus, Epstein Barr virus, and hepatitis virus are some of the viruses indicated in the etiology of viral myocarditis. The year in which maximum number of myocarditis (44%) was obtained in our study, coincided with the dengue epidemic [Figure 1]. The tissue reaction in myocarditis can be varied depending on the underlying etiology and associated pathology. Following infection, patients may develop lymphocytic, eosinophilic, neutrophilic or giant cell/granulomatous myocardial inflammation.[7] It can lead to infectious dialated cardiomyopathy, a disease frequently requiring cardiac transplantation. The predominant tissue response in our study was a mononuclear inflammatory infiltrate (70%) and in many of the cases (55) % there was positive history and serological tests favouring a

viral etiology [Figure 2].

Although acute viral myocarditis is frequently subclinical and recovery may be spontaneous, treatment of chronic myocarditis is currently unsatisfactory. Ongoing disease may be because of persistent virus in the heart or due to immunopathic attack.[8] Two proposed mechanisms are: molecular mimicry phenomenon resulting from cross reactivity of viral epitopes and some cardiac structures and second mechanism is the result of exposure of intracellular structures to immune system following virus induced myocyte damage.[7] With the advent of newer and better anti-viral drugs HIV has become a chronic treatable infection and thus another cause of myocarditis.

Diagnostic difficulty to the pathologist is due to the low sensitivity of detection of myocarditis in the endomyocardial samples, many a times specific symtoms will be absent.[8] Post mortem done in sudden deaths of young patents without a known cause may reveal the cause of death as myocarditis. Myocarditis is defined as an inflammatory process of the myocardium with associated cardiac dysfunction.[3]The Dallas criteria is the histopathological categorization useful to diagnose myocarditis and requires an inflammatory infiltrate and associated myocyte necrosis microscopically which are not characteristic of an ischemic event.[2] Borderline myocarditis will show inflammation but lack myocyte necrosis.[3] "Primary" (or Postviral) myocarditis forms the bulk of cases of myocarditis and thus forms an important category. Idiopathic or primary myocarditis is from myocardial inflammation due to persistent viral replication or autoimmune mechanism following an infection of viral etiology and includes several forms of myocarditis. These different forms are distinct in their clinical and pathological features which include fulminant, chronic active, eosinophilic, and giant cell myocarditis.[2]

Cardiac damage in viral myocarditis is damage to the heart by two mechanisms one due to direct viral cytopathicmyocyte injury and other by circulating autoantibodies causing immune-mediated injury of myocytes. Here myocardial injury has an initial period involving viremia. In the next phase viral proteins on the cell surface of cardiomyocytes leads to inflammation and myocarditis. Histopathological examination of myocardium at proper site usually reveals presence of inflammation and myocyte damage (Positive Dallas criteria). In the final (Resolution) phase, properly developed host defense mechanism removes the virus efficiently. Thus the myocardium moves towards healing and there will be decreasing number of inflammatory cells and increasing amounts of fibrosis depending on the extend of severity of the damage which occurred to the myocardium. Initial phases of myocarditis do not show significant myocardial inflammation to fit into Dallas criteria.[3]

In addition to histopathology using Dallas criteria and clinical presentation, for reaching a final diagnosis modern tools like immunohistochemistry, viral polymerase chain reaction, cardiac antibody assessment, and imaging results may become necessary.[2]

Association with conditions like HIV/AIDS, ischemia, inflammatory and immune disorders such as sarcoidosis and lupus erythematosus is attributed for myocarditis.[2] Inflammatory cardiomyopathy is the term used when myocarditis is seen in association with cardiac dysfunction. Myocarditis is an inflammatory disease of the myocardium and inflammatory myocardial disease is involved in the pathogenesis of dilated cardiomyopathy and other cardiomyopathies, eg, Chagas' disease, HIV, enterovirus, adenovirus, and cytomegalovirus.

Myocyte necrosis was detected in 98% of the cases in our study, however it was not found in two of the cases. In the absence of myocyte necrosis, it is considered as borderline cases of myocarditis. In such cases immunohistochemical studies can be done to detect the mononuclear cells that is by using CD 3 to detect the lymphocytes.[8,9] Woudstra et al has stressed the importance of using CD 45 also which might increase the sensitivity of detection of lymphocytic myocarditis.[10] The area of the myocardium from where the sample is taken is also detrimental in contributing to the diagnosis.

Sampling from the posterior wall of the left ventricle and using a combination of markers like CD 45, CD 3 and CD 68 has been recommended.[10]

Viral and bacterial infection related inflammatory cardiomyopathy has also been reported by some authors.[11] Kannel et al has suggested that 50% of sudden death in men and 64% in women occur without prior cardiac pathology.[12] Inflammatory cardiac pathology may aggravate the situation in persons with existing cardiac pathology. In some cases a combined etiology may be present. Our study showed evidence of coronary occlusion in 17.1% of cases only, but it was not critical. The extent of occlusion ranged between 25 to 60%.

In 70 % of cases myocarditis was seen to occur as a part of systemic involvement in our study and all such cases showed pathologic findings in other systems also. Since all cases of myocarditis received in our institution did not have specimens from other organs, it was difficult to correctly assess whether it is seen as a part of systemic viral infection or not. Cattelier et al has reported cardiovascular disease as the single most common generic cause of sudden and unexpected deaths.[13] Other studies have also stressed the role of cardiac pathology as an important cause of sudden death.[14,15] Evaluation of sudden deaths in young and middle aged individuals have revealed the role of cardiac diseases.[16] In our study we have tried to analyze the etiology, age distribution and related pathology in different age groups presenting with suspected myocarditis.

Immunohistochemical makers CD3 and CD 20 were done in fifteen cases and it showed positivity for CD3 which is a marker for T lymphocytes, thus helping to confirm the diagnosis in suspected cases of lymphocytic myocarditis.

	% of Myocarditis	M:F	Age Group	Mononuclear Cells	Myocte Necrosis	IHC Markers
Catelier et al	0.8%	1.8:1	41 yrs.	+	+	NA
Passarino et al	0.11 to 5.5%	1:1	20-39	+	+	NA
Woudstra et al	NA	NA	NA	+	+	CD 45,CD 3,CD 68
Ludden et al	NA	2.5:1	-	+	+	NA
Present study	0.2%	1.6:1	20-40	+	+	CD3,CD 20

Table 2. Comparison with Other Studies

[NA –not available]

Limitations

In mildly or partially autolysed specimens depending on the extent of autolysis there was loss of characteristic histopathological findings. This was found to interfere with the IHC results/findings. Serological details were not available in many of the cases, so a proper assessment of the etiological agents was not possible. IHC was done only in 0.2% of cases because in many of the prior cases the reagents did not work.

CONCLUSIONS

The gender predilection and age are comparable with other studies. The main histopathological features assessed in the myocardium were inflammatory infiltrate especially mononuclear cells and myocyte necrosis. [Table 2]

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