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DETECTION OF EARLY MYOCARDIAL INFARCTION DURING POSTMORTEM BY FLUORESCENT MICROSCOPIC EVALUATION

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ABSTRACT

Present clinical management of the patients sustaining traumatic injuries and thermal burns has resulted in their longer survival, but the clinical and pathological effects of these traumatic injuries over the myocardium have been largely neglected. It is speculated that certain factors like the inflammatory and degenerative lesions of heart, prolonged clinical course and the subsequent stress and strain may play role in hastening the death. In the present study, hospitalized cases of traumatic injuries and thermal burns brought for medico-legal autopsy were examined, with the purpose to find out the incidence, its significance and the extent of the myocardial lesions caused due to stress & strain following trauma. The myocardium of each case was examined morphologically, and by using fluorescence microscope utilizing the Acridine Orange (AO) staining method, to detect the presence of the myocardial lesions developing in the myocardium due to the effects of stress and strain following trauma.

KEYWORDS: Heart, Stress, Trauma, Acridine orange, Microscopy.

INTRODUCTION

Cardiac lesions in animals and human beings are known to cause by stress and strain leading to sudden deaths. Only few reports are noted in literature pertaining to the prognosis in cases of recent post-traumatic myocardial lesions [1]. It was hypothesized that these lesions, if

extensive, could contribute to or be the actual cause of death in such cases. In addition, these myocardial lesions are frequently missed during autopsy. The present study has been undertaken to evaluate the incidence, significance, and extent of myocardial lesion occurring due to trauma by fluorescent microscopic examination.

MATERIAL AND METHODS

Cases brought for Medico-Legal autopsy in mortuary of Apex Medical Institute. Cases selected were admitted in our institute or brought from private hospital for medicolegal autopsy, the cause of death was physical trauma, no direct cardiac injury evaluated clinically, no history of heart disease or coronary artery disease, age of the individuals below 65 years, minimum survival period of 4 hours following trauma. Details regarding treatment, history of incidences were collected from hospital records, documents supplied by the Investigating authority and relatives of deceased.

The specimens of heart were removed from the deceased body by Virchow's method of dissection [2,3] The heart thus removed, was rinsed immediately in physiological saline and heart weight was taken. The morphological examination of heart was carried out; colour of the myocardium pertaining to pale or hemorrhagic area or any other colour change was assessed for the presence of any recent ischemic area, the ventricles were cut into 1cm thick slices, parallel to atrio-ventricular groove by employing the Short-Axis Method of Cardiac Dissection (Transverse Slicing Method) as given by Ludwig, the presence of any sub-endocardial hemorrhages was noted. The slides prepared were stained by Acridine Orange for Fluorescent microscopic evaluation.

A standard fluorescent microscope, Nikon Optiphot Labophot having an epicscopic fluorescence attachment EF-D was used for Fluorescent Microscopy. The fluorescence of light green to grass green were interpreted as positive slides for evidence of ischemia / infarction and the slides with golden brown fluorescence were read as negative slides for normal myocardium.

RESULTS

As per table 1, out of the total 125 cases studied, 65.6% cases were males, 34.4% were females, and the female to male ratio is 1:1.90.

The age group ranged from 2 to 65 year with an average of 34.01 year. Most cases i.e. 29.6% cases belonged to the age group of 21 to 30 years whereas least of 0.8% cases were the age group of 1 to 10 years. In the age group of 31 to 40 years maximum numbers of males cases (25) were observed, whereas the maximum numbers of females (17 cases) were in the age group of 21-30 years.

The mechanical injuries were present in 59.2% cases and thermal injuries were present in 40.8% cases. From the table 2, it is seen that 87.83% males and 12.16% females had mechanical injuries and female to male ratio is 1:7.2.

From the table 3, it is seen that amongst the injuries by mechanical trauma; head injury was the leading cause of death accounting for 44.8% cases followed by head injury associated with injury to spine and spinal cord in 10.4%cases. Injury to spine and spinal cord was present in 2.7% cases. Head injury with blunt injury abdomen, blunt injury abdomen and crush injury lower limb were seen in 1.3% case each. Most of the cases of mechanical trauma are accidental, chiefly due to roadside traffic accidents, as per the police inquest, and circumstantial evidences.

The survival period of the cases examined varied from 4 hours to 3 months 20 days (110 days) having a median survival period of 81 days. From the table 4, it can be seen that maximum number of 32.2% cases survived for a period of less than 24 hours, whereas only one case survived for more than 1 month (3months 20 days).

The average heart weight in cases of males was 259.86 gm and in females was 240.52 gm. From the table 5, it can be seen that the heart weights, in both males and females were within the normal limits. On morphological examination of the heart, the colour of the myocardium pertaining to pale or hemorrhagic area or any other colour change was assessed for evaluating the presence of any recent ischemic area or an old infarct. Out of the total cases no case showed any evidence of change in the colour of the myocardium.

As shown in table 6 it is observed that out of the 125 cases examined, 20% cases were AO positive. Out of the total AO positive cases, 21.62% cases were of mechanical injury, and 17.64% cases were of thermal injury.

Table 7 shows the comparative analysis of the positive findings with their survival period. Out of 125 cases, examined subendocardial hemorrhages were present in 13.6% cases. Out of

these 17 cases, the subendocardial hemorrhages at 4 hour - 12 hour interval were seen in 46.6% cases; at 12 hour - 24 hour interval they were present in 35.7% cases; at 1 day - 2 day interval they were present in 27.2% cases and at 2 day - 3 day & 3 day – 4 day interval they were present in 6.2% & 6.6% cases each respectively. It can be seen that the incidence of positive findings of AO in the short survival period of 12 hours – 24 hours is 21.4% and that in the longer survival interval of 1 week to 2 week is 33.3%.

Table 1: Cases with reference to age and sex (n=125)

AGE GROUP (YEARS)	MALE	FEMALE	TOTAL	%
1-10	01	00	**01**	0.8%
11-20	09	12	**21**	16.8%
21-30	20	17	**37**	29.6%
31-40	25	09	**34**	27.2%
41-50	16	04	**20**	16%
51-60	06	01	**07**	5.6%
61-70	05	00	**05**	4%
Total	**82**	**43**	**125**	**100%**

Table 2: Cases according to the cause of death (n=125)

Cause of death	Number of cases					
	Male	%	Female	%	Total	%
Mechanical injury	65	87.83%	09	12.16%	74	59.2%
Thermal injury	17	33.33%	34	66.66%	51	40.8%
Total	82	65.6%	43	34.4%	125	100%

Table 3: Distribution of the cases of mechanical injury (n=74)

Cause of death	Male	Female	No of cases	% of cases
Head injury	51	05	**56**	**75.6%**
Head injury with injury to Spine & Spinal Cord	10	03	**13**	**17.5%**
Injury to Spine & Spinal Cord	01	01	**02**	**2.7%**
Head Injury with Blunt injury Abdomen	01	00	**01**	**1.3%**
Blunt injury Abdomen	01	00	**01**	**1.3%**
Crush injury lower limb	01	00	**01**	**1.3%**

Table 4: Cases according to survival period (n = 125)

Survival Period	Total	%
4-12 hours	15	12.0%
12-24 hours	14	11.2%
1-2 day	11	8.8%
2-3 day	16	12.8%
3-4 day	15	12.0%
4-5 day	14	11.2%
5-6 day	13	10.4%
6-7 day	08	6.4%
1week- 2week	15	12.0%
2week- 3week	03	2.4%
3week-1month	00	--
1month and above*	01	0.8%

Table 5: Cases according to the external morphological examination of the heart (n=125)

Age Group (Years)	Average Heart Weight (gms)		Colour of Myocardium
	Male	Female	
1-10	220	-	N
11-20	240	227.7	N
21-30	243	230.5	N
31-40	278	234.4	N
41-50	270	250	N
51-60	280	260	N
61-70	288	-	N
Average	259.86	240.52	-

Table 6: Findings on fluorescent microscopic evaluation by acridine orange (ao) staining method (n=125)

Cause of death	AO Positive (Grass green fluorescence)	%	AO Negative (Golden brown fluorescence)	%
Mechanical injury	16	21.62%	58	78.37%
Thermal injury	09	17.64%	42	82.35%
Total	**25**	**20%**	**100**	**80%**

Table 7: Comparative analysis of the positive findings of the methods employed in this study with the duration of survival of the cases

Survival Period	Total cases	Subendocardial Hemorrhages	AO Positive
4-12 hours	15	07 (**46.6**%)	02 (**13.3**%)
12-24 hours	14	05 (**35.7**%)	03 (**21.4**%)
1-2 day	11	03 (**27.2**%)	02 (**18.1**%)
2-3 day	16	01 (**6.2**%)	02 (**12.5**%)
3-4 day	15	01(**6.6**%)	02 (**13.3**%)
4-5 day	14	00	04 (**28.5**%)
5-6 day	13	00	02 (**15.3**%)
6-7 day	08	00	01 (**12.5**%)
1week- 2week	15	00	05 (**33.3**%)
2week-3week	03	00	01 (**100**%)
3week-1month	-	-	-
1month and above	01	00	01 (**100**%)
Total	**125**	**17**	**25**

DISCUSSION

Present clinical management of the patients sustaining traumatic injuries and thermal burns has resulted in their longer survival, but the clinical and pathological effects of these traumatic injuries over the myocardium have been largely neglected. It is speculated that certain factors like the inflammatory and degenerative lesions of heart, prolonged clinical course and the subsequent stress and strain may play role in hastening the death. Studies related to the effect of trauma on myocardium have centered primarily upon the assessment of pathologic damage caused due to the direct trauma on the myocardium but few had tried to address the effect of stress and strain owing to trauma on the heart.

As compared to other age groups, the higher involvement of the males in 2nd and 3rd decade of life is due to more active outdoor activities, thereby increasing the possibility of fatal trauma. Whereas the higher association of females in 2nd decade can be attributed to the fact that most of the females in this age group are newly married and remain indoors. Also, they are more inclined to be subjected to the risk of fatal thermal injuries due to more involvement in household activities like cooking. Connor RCR [4] corroborates with this and had maximum number of cases in 2nd & 3rd decade.

Males were more involved in fatal mechanical injuries than females as the males are more actively involved in outdoor activities as compared to females. 66.6% females and 33.3% males had burn injuries and female to male ratio is 1:0.5. Females are more involved in

accidental thermal injury as compared to males, on account of their loose garments catching fire, while doing their household activities like cooking etc. Besides this, most of the cases are suicidal as per their dying declaration and police inquest, along with the circumstantial evidences.

Analyzing the period of survival, as illustrated in table 4, it has been observed that the cases within the survival period of 24 hours had multiple trauma, whereas the cases having the survival period of more than 2 days had sustained comparatively less traumatic injuries. Mortality from trauma appears in "tri-model" distribution. Deaths occurred either immediately, or within the first few hours or much later (days, or even weeks) after the trauma. [5] A better understanding of the pathophysiology of trauma and improvements in the early management of such traumatic patients seems to have resulted in reduction in the early deaths. Evaluating the period of survival after the time of injury, maximum number of cases i.e. 12.8% cases survived for 2 to 3 days. These were followed by the survival period of 4 to 12 hours and 3 to 4 days in 12 % cases each. In 0.8% cases the maximum survival period was found to be 3 months 20 days. The duration of survival of the cases studied by Rajs J [6], Yoshida K et al [7], corroborates with the present study. The average length of survival for the present study was 81 days; the length of survival after the terminal ictus does not correspond with the study of Connor RCR [4,8] which was 8 days and 2.8 days respectively, much shorter than in this study.

Gross external examination of the hearts showed no significant abnormality in the form of heart weight or any colour changes and this finding corroborates with Connor RCR. [8] However the present finding does not corroborate with the study of Rajs J &Jakobsson S [9] and Joshi VV [10] which have mentioned the presence of epicardial & endocardial fibrosis and severe cardiomegaly respectively on external examination. This finding might be due to longer interval between injury and death (1 yr-10 yr) of the cases studied by the authors.

The traumatic lesions especially the head injury, the spinal cord injuries and burn injuries produce hypoxia, hypotension, tachycardia, anemia and other deleterious effects particularly ventricular fibrillation. These factors in the presence of an inadequate perfusion may interact to produce subendocardial ischemia and necrosis resulting in subendocardial hemorrhages, even if the coronary arteries are not occluded. In case of mechanical injury the incidence of above mentioned factors are more as compared to thermal injury and hence these subendocardial hemorrhages are present in more cases in mechanical injury as compared to

thermal injury. Out of 125 cases examined, the myocardial lesions were found in 25 cases, out of which 19.51% were males and 20.53% were females. The female to male ratio of the positive cases was 1:1.77. No significant gender difference was noticed in the present study. This finding corroborates with the finding of Joshi VV. [10] But it does not corroborate with the findings of Tung P et al [11], Homma & Clarke [12] and Kher et al [13] where gender difference has been noted. Tung P et al [11] and Homma & Clarke [12] noted more preponderance of females for myocardial lesions following trauma and attributed it to the difference in catecholamine secretion in the two genders. According to them, in females, higher catecholamine spill-over occurs. Kher et al [13] recorded less number of female involvements and the reason was ascribed to presence of estrogen in females which is cardio-protective.

In the present study, subendocardial hemorrhages were found in 13.6% cases out of 125 cases mostly over the outflow tract of left ventricle. The findings in the present study corroborates with Jovan Rajs [6], Connor RCR [4] and Harruff RC [14] in which subendocardial hemorrhages were reported in 15%, in 18% and in 16% cases respectively. The presence of these subendocardial hemorrhages corroborates with the studies of Chang and Hackel [15], Johansson G et al [16], Hackel et al [17], Yoshida K et al [7] who have mentioned the presence of these hemorrhages in animals and humans subjected to hemorrhagic shock and various restraint stresses. This finding does not corroborate with the findings of Varga & Szabo [18] who had found presence of subendocardial hemorrhages in all the 72 cases (100%). This may be due to small sample size and the shorter duration of survival of the cases studied by them.

In the present study it has been found that the incidence of the myocardial lesions detected by AO method increases with the increase in the survival period of the deceased. This corroborates with findings of Sharma VN & Barar FSK. [18] On analyzing these findings, it can be observed that the incidence of subendocardial hemorrhages decreases with the increase in the survival time. This is because as the survival period increases, these subendocardial hemorrhages starts fading up and become macroscopically pale and discoloured.

In the present study it was observed that the Acridine orange (AO) staining methods is good staining method, for detecting the early myocardial changes caused due to stress and strain following trauma; corroborates with the findings of Hammermeister K.E & Reichenbach DD. [19] However, Boor PJ & Reynolds ES [20], Siegel RJ & Fishbein MC [21] does not corroborate

with the findings of the present study. This might be due to the fact that, the authors have studied only the clinically diagnosed cases of myocardial ischemia.

The observation of the present study that the Acridine orange (AO) staining method is superior and more sensitive for detecting the myocardial lesions having a short survival interval corroborates with findings of Knight B [22], Sahai VB & Knight B [23], Lachica E et al.[24]

The incidence of detection of the myocardial lesions by AO increases with the increase in the survival period of the deceased. Acridine Orange (AO) staining method is a very sensitive method and is capable to detect the myocardial ischemic or hypoxic lesions as early as 2 to 3 hours. Thus, the fluorescent microscopic examination by Acridine Orange (AO) staining method can be used to determine the myocardial lesions occurring due to the stress and strain following trauma.

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