Indian Journal of Medicine and Healthcare

Post mortem, study of heart in cases of sudden cardiac death using acridine orange fluorescence and haematoxylin and eosin stain

Hetal Gohel[1*], Nandini Desai[2], Sujata Tripathi[3], Bansidhar Gupta[4] and Pravina Santwani[5]
*[*12,3&5]Department of Pathology, M.P. Shah Medical College, Jamnagar, Pin code -361008, Gujarat, India..*
[4]Department of Forensic Medicine, M.P. Shah Medical College, Jamnagar, Pin code -361008, Gujarat, India.
drhetalgohel99@gmail.com[*1];toshakhushi@yahoo.com[2]; drsujata.tripathi@gmail.com[3]; bdgujn@yahoo.com[4]; pravinasantwani@yahoo.com[5]

Abstract

Background: Myocardial infarction is the most common cause of death for which the largest numbers of autopsies are done.

Aim: A clinical study was done to determine those numerically smaller but medico-legally more important cases in which some unnatural event like a road accident, is suspected to have been precipitated by acute myocardial ischemia. The likelihood of identifying an early infarct depends on the mechanism of the terminal cardiac failure.

Materials and method: A histochemical study on early ischemic changes in 100 hearts which can detect the healthy as well as the infarcted myocardium due to enzymatic changes in the myocardium, using haematoxylin and eosin as a routine stain and acridine orange as a fluorescent stain, the latter can detect infarcts of even 2 hours of age to assess the actual age of the infarct and confirmation of myocardial infarction as the cause of demise was done.

Results: 37% of the cases were seen in the 6th decade followed by 4th & 5th decade, males were more affected, the male to female ratio being 11.5:1. 75% of the cases showed grade 2 and grade 3 stenosis of coronary artery. Majority of the hearts were within normal weight range showing absence of chronicity of disease.

Conclusion: Sudden death due to myocardial infarction is still the disease of middle age with increasing incidence in younger age groups, acridine orange is the earliest detector of infarction and degree of stenosis is directly related with the age factor most common cause for that being atherosclerosis**.**

Keywords: Acridine orange, Early infarct, Fluorescent microscopy.

Introduction

Ischemic heart disease (IHD) is the leading cause of death worldwide for both men and women. IHD is the generic designation for a group of pathophysiologically related syndromes resulting from myocardial ischemia-an imbalance between the supply (perfusion) and demand of heart for oxygenated blood. Ischemia brings not only an insufficiency of oxygen, but also reduces the availability of nutrients and removal of metabolites. In more than 90% the cause of myocardial infarction (MI) is reduced blood flow due to obstructive atherosclerotic lesion in the coronaries thus IHD is also termed as coronary artery disease (CAD) or coronary heart disease (CHD) (Ramzi *et al.,* 2010).

CAD is by far the most frequent cause of sudden and unexpected deaths which constitute a significant portion of autopsies conducted by forensic pathologists in our country, since these deaths appear in apparently healthy without any diagnosed diseases it can be attributed to the cause of death, or the period of illness before the supervening of death is so short that the disease cannot be diagnosed early (Strong & Mcgill, 1962).

The realization of the need for establishing the diagnosis of myocardial infarction in the initial 8 hours, where definite evidence of infarction is lacking has propelled a number of studies on the histochemical, electron microscopic and fluorescent microscopic changes. Also many times significant stenosis of coronary arteries is encountered in deaths due to some other natural disease in these cases the query always arises whether myocardial ischemia might have contributed to death.

With these considerations, it was thought worthwhile to study myocardial ischemic changes, not only by routine staining methods but with fluorescent microscopy also.

The present study has been carried out using hematoxylin and eosin as a routine staining method and acridine orange as a fluorescent marker on dead myocardium to separate it from viable myocardium to assess the actual age of the infarct and confirm myocardial infarction as the only cause of death.

Materials and methods

The present study was carried at the Department of pathology, during the period of Aug. 2009 to Aug. 2011.Detailed history and post-mortem findings of heart of all cases were collected.

Selection of cases: Totally 100 cases were studied. Post mortem (PM) study was carried out in clinically suspected / confirmed cases of MI. In addition 3 cases (1 case of fall from height, 1 case of dehydration and 1 case of hypertrophic obstructive cardiomyopathy) of sudden death due to other causes were also included in the study as negative control.

All cases were subjected to acridine orange staining whether these cases showed positive result on H&E stain or not. Total 15 cases of paraffin embedded block of grossly and histological proven cases of MI were taken as positive controls and for standardization of the acridine orange fluorescence study. A detailed history was taken pertaining to the circumstances of death with special reference to any signs and symptoms suggestive of myocardial ischemia like chest pain, sweating, collapse and shock, and details regarding the time of onset of illness and to the eventual event of death, and also hospital records were procured wherever possible.

The weight of the heart was recorded. Hearts obtained from the test cases were subjected to meticulous gross examination of all three coronary vessels and their major branches by serial transverse sectioning of vessel to note the degree of calcification, stenosis, and presence of thrombus or hemorrhage into an atheromatous plaque. This was done by using a sharp scalpel and cutting transverse sections of the coronaries and their main branches at 3 mm intervals. Arteries examined included the left main, the left circumflex, the anterior descending, the right main and the posterior descending.

Routine microscopic examination by H & E stain as well as fluorescence study by acridine orange staining of suspected infarct or peripheral area of visible fresh infarct were undertaken

Acridine orange fluorescence study

Staining procedure: 1. 1% solution of Acridine orange is prepared by dissolving 1gm of the powder in 100 ml phosphate buffer of pH7.2, Paraffin embedded sections are brought to water, 3. Staining with acridine orange solution is effected for 3-5 seconds, the slides being continuously adjusted during this time to avoided non-specific deposition of acridine orange, 4. The stained sectioned are repeatedly rinsed in phosphate buffer for 10-15 minutes using 2-3 changes of buffer solution. This resulted in washing away of non-specific deposits of acridine orange. 5. The sections are then mounted in buffer solution and viewed under UV fluorescent microscope immediately. At the end of the staining, the sections attained moderately dark brown colour visible to the naked eye.

Interpretation: Examination under fluorescent microscope using low power magnification revealed infarcted tissue by its grass green colour. The intensity of green colour is directly proportional to the severity of damage - established scar tissue being brightest; early infarcts showing only light green fluorescence (Fig.1). Intermediate grades of infarcts showed different shades of green colour according to the severity (duration of process) of the process (Fig. 2 & 3); while the normal myocardium showed golden brown fluorescence. Considerable difficulty was experienced in picking up the fine tinge of light green fluorescence of early infarcts until experience was gained.

Haematoxylin and eosin stain

The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized haematoxylin.

ISSN: 2278 - 2966 (Print), ISSN: 2278- 3334 (Online)
Vol.1 No.5 (Aug. 2012)

Fig.1 Early Infract ,the recent infarct is seen as light green colour (Acridine Orange, X400).

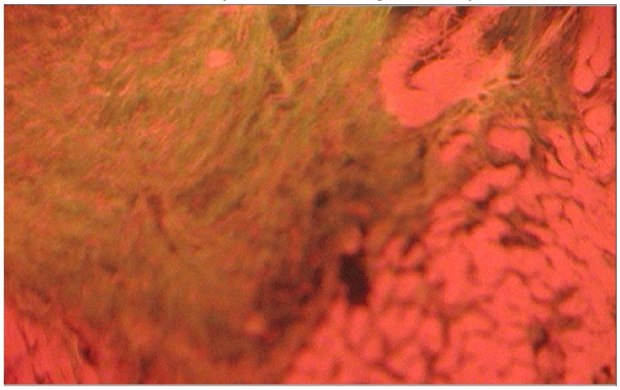


Fig.2 Intermediate infarct, different shades of green are seen based on the age of infarction. (Acridine Orange, X400).

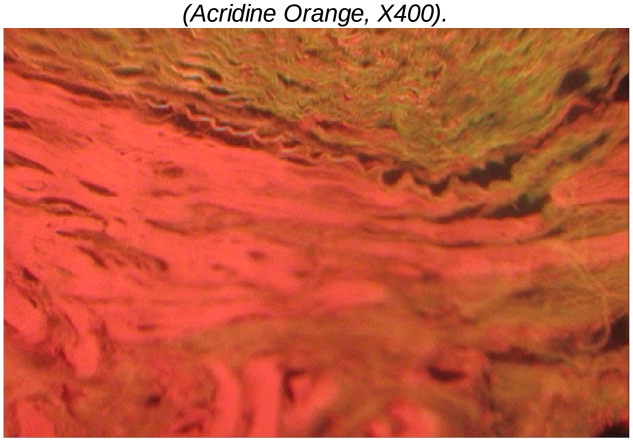


Fig. 3 Old Infarct, grass green colour of the old infarct seen. (Acridine Orange, X400).

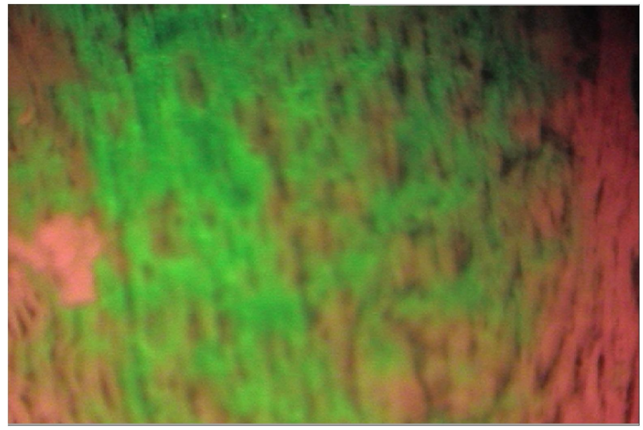
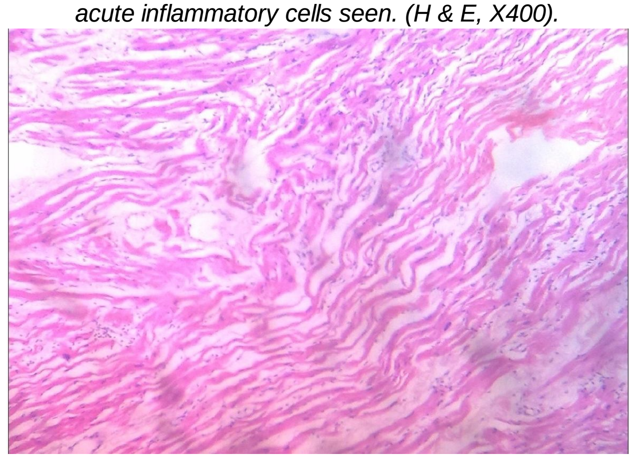


Fig. 4 Acute myocardial infarction, waviness of fibres and acute inflammatory cells seen. (H & E, X400).



These colours nuclei of cells (and a few other objects, such as keratohyaline granules) blue. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colors other, eosinophilic structures in various shades of red, pink and orange(Fig.4).

Results

The study comprised of 100 cases that were brought to the Department of Forensic Medicine, for Medico legal autopsy during the period of Aug. 2009 to Aug. 2011. Cases showing signs of decomposition were not considered. Cases of sudden unexpected deaths with history suggestive of heart disease were especially included. Detailed histories regarding the circumstances leading to death, any past history of myocardial infarction, or symptoms suggestive of heart disease like, breathlessness, chest pain, collapse, were obtained from the relatives, inquest papers and wherever possible from the hospital records. In none of the cases ante, mortem diagnosis of myocardial infarction was made (that presented as sudden death). Either the patient was found dead or was declared in the hospital as “brought dead”.

As per table 1, 92% of cases show positive results by acridine orange test. The lowest age group in which 66.66% results were detected by acridine orange is 11-20 years. 90% to 100% acridine orange positivity was obtained in age group range 21-80 years.

Myocardial infarction developing in younger age is severe enough to cause sudden death which shows low post myocardial infarction survival time and can be very well appreciated by acridine orange. (Fig. 5)

ISSN: 2278 - 2966 (Print), ISSN: 2278- 3334 (Online)
Vol.1 No.5 (Aug. 2012)

Fig.5 Graph showing age distribution and changes of infract with

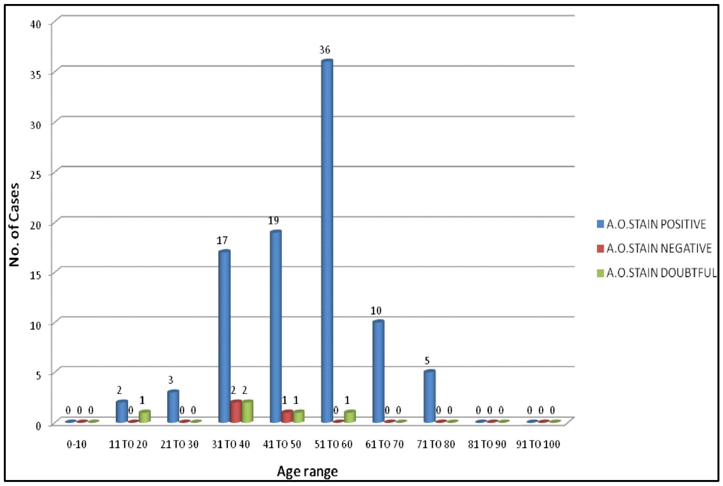
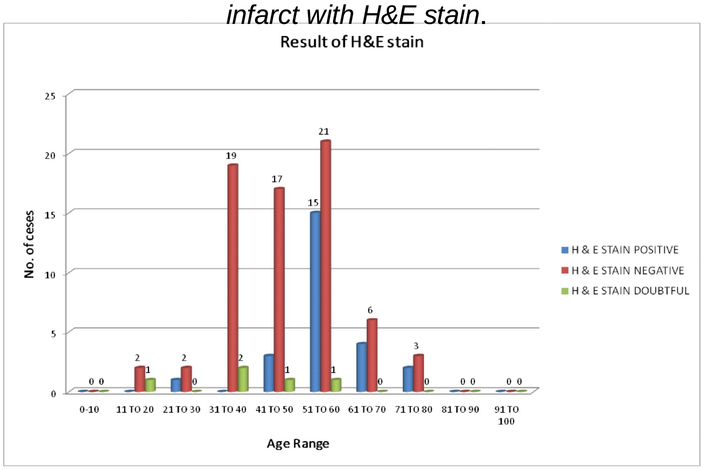


Fig. 6 Graph showing age distribution and changes of infract with H&E stain.



As per Table 2, only 25 cases out of 100 were detected by H&E stain. 40% of result was obtained in age group 51-80 years indicates low sensitivity of H & E stain in selected sample type and post myocardial infarction survival duration is longer in middle to older age group.(Fig.6)

As per table 3, maximum (37%) cases were seen in 6th decade followed by 4th and 5th decade. Three cases of cardiac death were seen in younger age (2nd decade). All three young deceased were males. Amongst them history of sudden death, vomiting and breathlessness was sought. As per table 4, maximum males were affected. The ratio of M: F is 11.5:1

As per table 5, cases with traceable history related with myocardial infarction were maximum (62%), followed by sudden death (30%). Sudden death is one of the common presentations in acute myocardial infarction.

As per Table 6, maximum (75%) cases were showing grade 2 ° & grade 3° stenosis of coronary artery. Majority of hearts were within normal range of weight indicates absence of chronicity of coronary heart disease.

Discussion

The absence of gross as well as microscopic changes before appearance of neutrophil at the scene of infarct, which is estimated to take minimum of 6-8 hours in term of post-infarction survival time, has been a major hurdle in establishing the cause of death in cases of early death due to myocardial infarction. The present study of myocardial infarction was evaluated in 100 cases with fluorescence microscopy and hematoxylin and eosin stain. The observation and their correlation with other authors are discussed in following paragraphs.

The present study was very well correlated with Lachica et al.(1988) who had found that acridine orange staining method is more sensitive than H & E stain and also useful and reliable to confirm the diagnosis of myocardial infarction when it is doubtful or negative by H & E stain. The present study gave precise results with acridine orange staining which concluded that more number of fresh infarcts had been included in present study with satisfactory collection of sample size and type. Above cited table showed good correlation of other studies with present study except Chen et al. (1992) may be due to different geographical region and environmental factors.

Incidence of myocardial infarction in younger age group:

In present study, youngest male to show infarction is 19 years; the youngest female is 26 years. Callegari (1986) demonstrated the positive correlation between MI and hypercholesterolemia in the younger groups in both sexes, but mainly in female. The strong association of hyperlipidemia and MI in the younger group and in female, documented, confirmed the importance of hypercholesterolemia and

Table 1.Result of Acridine orange

Age Range	Total cases	Positive cases		Negative cases	Doubtful cases
		No	%		
0-10	0	0	0%	0	0
11-20	3	2	66.66%	0	1
21-30	3	3	100%	0	0
31-40	21	17	81%	2	2
41-50	21	19	90.47%	1	1
51-60	37	36	97.29%	0	1
61-70	10	10	100%	0	0
71-80	5	5	100%	0	0
81-90	0	0	0%	0	0
91-100	0	0	0%	0	0
Total	100	92	92%	3	5

Table 2. Result of H&E stain

Age range	Total cases	Positive cases		Negative cases	Doubtful cases
		No	%		
0-10	0	0	0%	0	0
11-20	3	0	0%	2	1
21-30	3	1	33.33%	2	0
31-40	21	0	0%	19	2
41-50	21	3	14.28%	17	1
51-60	37	15	40.54%	21	1
61-70	10	4	40%	6	0
71-80	5	2	40%	3	0
81-90	0	0	0%	0	0
91-100	0	0	0%	0	0
Total	100	25	25%	70	5

hypertriglyceridemia as a risk factor in the early development of coronary heart disease.

Wayne Falcone (2004) found the frequency of acute myocardial infarction in person's ≤45 years of age is relatively uncommon, particular in women. Acute myocardial infarction occurred in 303 woman (11%) and in 2488 men (89%) <45 years of age. Of the 303 women, only 5 (2%) were stated to be ≤25 years of age. acute myocardial infarction in the 20s is extremely rare. A number of studies have demonstrated that the number of atherosclerotic risk factors in young patients with acute myocardial infarction is considerably larger than in older patients with acute myocardial infarction.

Arnold and Moodie (1993) described 32 women ≤30 years of age referred to the Cleveland Clinic for evaluation of coronary artery disease. Of the 32 patients, 22 had an acute myocardial infarction.

All above studies correlated with findings of the present study, which indicated very low incidence of myocardial infarction in young female**.**

In present study, the incidence of sudden myocardial infarction death was higher in males than females which is correlated with the studies reported except two studies (WHO scientific group and Alicja et al., (2010) where sample size was larger and may have included all the cases of myocardial infarction (including chronic ischemic heart disease) Lina & Sidir (2006), Radhakrishnan, (2009) reported that oestrogen has cardioprotective effects against acute injury through a variety of complex mechanisms. Strong and McGill, (1962) also found coronary artery lesions to be more in males than females.

Incidence and severity of atherosclerotic lesions and stenosis in myocardial infarction:

The present study also indicated a strong correlation between the arterial lesions of coronary atherosclerosis and ischemic heart disease in that both the incidence and severity of atherosclerotic lesions were greater in the hearts that showed ischemic changes. It was seen that the incidence of myocardial ischemia increased with the increasing grade of stenosis.

This agrees with Strong and McGill (1962) who opine that this correlation between coronary lesions and fatal ischemic heart disease suggest that the modern epidemic is based primarily on atherosclerotic lesions rather than a terminal episode such as thrombosis, and that the severity of coronary lesions is the determining factor in morbidity and mortality from ischemic heart disease.

Conclusion

Pursuing the aims of the study, following conclusions are made:

Table 3. Age wise distribution of cases

Age group	Males		Females		Total	
	N0	%	No	%	No	%
0-10	0	0%	0	0%	00	0%
11-20	3	100%	0	0%	03	3%
21-30	2	66.7%	1	33.3%	03	3%
31-40	19	90.5%	2	9.5%	21	21%
41-50	21	100%	0	0%	21	21%
51-60	33	89.2%	4	10.8%	37	37%
61-70	10	100%	0	0%	10	10%
71-80	4	80%	1	20%	05	5%
81-90	0	0%	0	0%	00	0%
91-100	0	0%	0	0%	00	0%
Total	92	92%	8	8%	100	100%

Table 4. Distribution of cases according to gender

Sex	No. of cases	
	Number	Percentage
Male	92	92%
Female	08	8%
Total	100	100%

Table 5. Distribution of cases according to history (n =100)

History	Cases	
	Number	Percentage
Sudden death	30	30%
Fall from height	01	1%
Gas & acidity	02	2%
Suspected myocardial infarction	62	62%
History was not available	05 (5%)	5%
Total	100	100%

Table 6. Distribution of cases according to grading of stenosis

Grading	Cases	
	Number	Percentage
Grade 0	04	4%
Grade 1	05	5%
Grade 2	36	36%
Grade 3	39	39%
Grade 4	16	16%
Total	100	100%

- Acridine orange detects youngest infarction (upto 2 hrs of age). The acridine orange fluorescence study interpretation needs some experience to 'visualize' especially early infarct.
- The non-availability of UV fluorescent microscope might be a limiting factor. While carrying out this type of study to minimize false results, selection of proper sample type is very important.
- Fluorescent stains may be used when available in all sudden deaths said to be due to heart attack in medico legal cases with good success rate to get the diagnosis, which is otherwise not available grossly, as well as on routine histopathology examination by H & E stains.
- An attempt should be made to establish fluorescent microscopy at all the hospitals including primary centers where the post-mortems are conducted.
- All medical teaching institutes should be given the privilege to start fluorescent microscopy at the earliest.
- Sudden death due to acute myocardial infarction is still the disease of middle age group showing rising incidence in younger ones with sporadic etiological factors.

Table 7.Distribution of cases according to weight of the heart

Weight of heart (gm)	Cases	
	Number	Percentage
200-300	56	56%
301-400	41	41%
401-500	01	1%
501-600	01	1%
>600	01	1%
Total	100	100%

- Males are more prone to develop acute infarction than females.
- Degree of stenosis is directly related with age factor and common cause for that is atherosclerosis.

Table 8. Comparison of results of Acridine orange and H & E stain

Study	H&E			Acridine orange		
	Postive (%)	Negative (%)	Doubtful (%)	Positive (%)	Negative (%)	Doubtful (%)
Lchica,(1988)	61.9	28.6	9.5	75	15	10
Present study	25	70	5	92	3	5

Table 9.Comparison of age incidence of myocardial infarction		
Author		Majority of MI found in age group/ Mean age
Chen & Huang(1992)		30-49 years
Peter et al.(2003)		40-60 years
Jens et al.(2002)		67 ±6
Jalal & Hasan(2005)	1991	Mean age 59.18
	2001	Mean age 59.1
Brian et al. (1986)		46-70 years
Alicja et al. (2010)		56-60 years
WHO scientific group(1970)		40-59 years
Liaqat ali et al.(2011)		56.2±12.7 years
Bhalla et al. (2006)		57±5.4
Present study		51-60 Years

Table 10. Comparison of gender difference					
Author		Male		Female	
		Number	Percentage	Number	Percentage
Chen & Huang (1992)		74 cases	83%	15cases	16.9%
WHO scientific group(1970)		312cases	77.2%	72cases	49.6%
Liaqatali et al.(2011)		228 cases	74.3%	79cases	25.7%
Bhalla et al.(2006)		-	64.2%	-	35.8%
Jalal & Hasan(2005)	1991	95 cases	77.9%	27 cases	22.1%
	2001	243 cases	69.6%	106 cases	30.4%
Craing & Wiliam(2004)		31 cases	83.8%	6 cases	16.2%
Alicja et al.(2010)		692 cases	65.7%	361cases	34.3%
Present study		92 cases	92%	08 cases	8%

References

1. Alicja B, Elzbieta G, Jadwiga S, Agata S, Piotr V and Jerzy K (2010) Work related risk factors of myocardial infarction. IJOMEH.23(3), 255-265.
2. Arnold AZ and Moodie DS (1993) Coronary artery disease in young women: Risk factor analysis and long term follow up. *Cleve. Clin. J. Med.* 60,393-398.
3. Bhalla A, Sachdev A, Lehl SS and Singh R (2006) Aging and circadian variation in
4. cardiovascular events. *Singapore Med. J.* 47(4), 305-308.
5. Brian D, Hoit, Elizabeth AG, Hartmut H, Alan AM, Howard D, et al. (1986) Myocardial infarction in young patients: an analysis by age subsets. *J. Am. Heart Assoc.* 74(4), 712-721.
6. Callegari S, Andreoli CS, Arsenio L and Strata A (1986) Retrospective study on 778 patient with Acute myocardial infarct: Relation between sex, age and blood lipid picture.57 (5-6), 141-153.
7. Craig SC and William CR (2004) Clinical and necropsy finding in patients with calcified myocardial infarcts. Proc. *Bayl. Univ. Med. Cont.*17 (4), 420-424.
8. Jalal SS and Hasan MK (2005) Myocardial infarction: Factors influencing age of onset and comparision with past decade. Iranian heart Journal.2005; 6(1-2):11-16.
9. Jens PH, Guy SR, Steven JJ, Susan AW, Jill MK and Veronique LR (2002) Longitudinal trends in severity of acute myocardial infarction: A population study in Olmsted county, Minnesota. *Am. J. Epidemiol.*156, 246-253.
10. Lachica E, Villanueva E and luna A (1988) Comparision of different techniques for the postmortem diagnosis of myocardial infarction. *Foren. Sci. Intl.* 38, 21-26.
11. Liaqatali, Abdul Rehmanabid, Imtiaz Ahmed, Nusrat Niaz et al. (2011) Myocardial infarction in diabetics; Circadian periodicity in the onset of Acute ST segment elevation. *Profesional Med. J.* 2011; 18(2), 269-274.
12. Lina SK and Sidir PK, Cellular mechanism underlying the cardiovascular action of oestrogen (2006) *Clin. Sci.* (Lond). 111:107-118.
13. WHO (1970) The pathological diagnosis of acute ischemic heart disease, World Health Organisation. *Techn. Rep. Ser.* 441.
14. Peter Nyamu, Mullasari SA, Peter KJ, Lakshmi V and Nang AS (2003) The prevalence and clinical profile of angiographic coronary ectasia. *Asi. Cardiovasc. Thorac.* 11, 122-126.
15. Radhakrishnan R (2009) Risk factors for myocardial infarction in women and men. *Eur. Heart J.* 30(8), 1012-1013.
16. Ramzi S Cotran, Vinay kumar, Abbas Fausto, Aster Robbin's (2010) Pathological

ISSN: 2278 - 2966 (Print), ISSN: 2278- 3334 (Online)
Vol.1 No.5 (Aug. 2012)

basis of disease. Eighth edition, PA, USA, *Elsevier*. 529-587.

17. Strong JP and Mc gill HC (1962) The Natural history of coronary atherosclerosis. *Am. J. Pathol.* 40, 37-49.
18. Wayne M Falcone, Paul AG, William CR (2004) Acute Myocardial infarction at 25 year of age. *Proc. Bayl. Univ. Med. Cent.* 17(3), 363-365.
19. Xinshen Chen and Guangzhao Huang (1992) A pathological study of sudden coronary death in china: report of 89 autopsy cases, *Foren. Sci. Intl.* 57, 129-137.