Evaluation of apoptosis in myocardial injury in cases of medicolegal death

Ozlem Erel¹*, Berk Gun², Canten Tataroğlu³, Musa Dirlik¹

Abstract: The most common cause of sudden unexpected deaths is cardiovascular system diseases. Ischemic heart injuries take the biggest part in this group. Cardiac diseases such as coronary artery disease and myocardial infarction play a significant role in forensic medicine practice due to being very common and fatal. Therefore, many methods are being tried in postmortem diagnosis and reliability and validity studies are performed for these methods.

In this study, we aimed to investigate the postmortem diagnostic features of apoptosis in the myocytes in myocardial injury. For this reason, apoptosis conditions were evaluated using terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL), Bcl-2, Fas, and p53 immunohistochemical staining methods in the myocardial tissues of 43 medicolegal autopsies.

The cases that have 75% or more stenosis in at least one of the coronary arteries and that had been diagnosed with myocardial infarction apoptosis markers are stained in a higher rate. In the cases that have 50% or more stenosis in at least one coronary artery, Fas and bax staining were found as statistically significant. Apoptosis may be helpful in diagnosing the cases with myocardial injury such as acute myocardial infarction and Bcl-2, bax, Fas, p53 markers and TUNEL staining method may be used for his purpose. We think that these methods may be insufficient due to the low specificity and suggest supporting them with other methods.

Key Words: myocardial infarction, apoptosis, postmortem, autopsy, forensic medicine, pathology.

It is estimated that there are nearly 60-80 thousand forensic deaths per year in Turkey [1]. Due to the definition by World Health Organization (WHO); sudden death is the death that occurs within 24 hours of symptom onset. The majority of deaths due to myocardial infarction (MI) occur in the first hours. However, in the first six hours macroscopic and microscopic changes do not occur [2-4].

It is possible that apoptosis occurs in early stages right after the onset of myocardial ischemia and is the biggest cause of cell death after coronary occlusion in the first couple hours [5]. The regulation of apoptosis is provided by specific proteins such as Bcl-2 / Bax gene family that connects the original death signals to the final execution program [6, 7]. Bcl-2, Fas and Bax expressions were increased in ischemic myocardium [8-15], whereas mild ischemia leads to apoptosis in myocytes as in all cells, severe ischemia results in necrosis [8, 16]. In myocardial infarction, both types of cell death are observed together in the infarction site [8]. In the myocardial samples that are taken from the patients who died of acute myocardial infarction (AMI), in addition to the significant necrosis, one group of myocytes undergo apoptosis [17]. Apoptotic myocytes are most notably found on the borderline of

1) Adnan Menderes University Faculty of Medicine, Department of Forensic Medicine, Aydin, Turkey
* Corresponding author: Ass.Prof.MD., Adnan Menderes University Faculty of Medicine, Department of Forensic Medicine, Aydin, Turkey, Email: overel@yahoo.com
2) Department of Forensic Medicine, Ministry of Justice, Izmir, Turkey
3) Adnan Menderes University Faculty of Medicine, Department of Pathology, Aydin, Turkey
the last infarction whereas very few apoptotic cells were found in the non-infarcted distant myocardium areas [5, 17-19]. Apoptosis is shown histological on the infarction tissue both in the early and the late phases [20]. A heterogeneous image is seen histological in the infarction areas, whereas the apoptosis was most intensively seen in the area that limits the infarction [8]. Even though the artery responsible for the infarction is open, it was detected that the apoptosis intensity in the peri-infarct zone continued [21].

Bcl-2, which is an apoptosis inhibitor, is not expressed on non-infarcted myocardial tissue [22], it is expressed in the cardiomyocytes that surround the infarction areas shortly after the onset of infarction. However, there was no bcl-2 found in the infarction area itself. Bax is a member of bcl-2 family and it accelerates apoptosis when overly expressed. Also, it contributes to the death suppressing activity of bcl-2 [23]. In contrast to bcl-2, bax has a low basal expression in the hearts of the people who do not have cardiac diseases [24].

In acute myocardial infarction (AMI), the levels of soluble Fas (sFas: an apoptosis inhibitor) are elevated, whereas the sFas ligand (an apoptosis inducer) levels do not change [25]. The circulating sFas levels increase significantly in acute MI compared to the old MI. sFas levels increase independently of the infarction size in AMI [26].

Except for the ischemic hearts and hearts with failure, cardiac arrhythmias are an important disease entity that apoptosis plays a role in their pathogenesis [11, 13, 27, 28].

Because of high sensitivity in unexpected deaths, use of apoptosis as a routine marker was investigated in this study. Our aim was to define the importance of apoptosis markers postmortem diagnosis.

MATERIALS AND METHOD

Forty-three medicolegal autopsies were randomly selected among a total of 461 medicolegal deaths in Aydın city during a period from January 1st 2010 to March 1st 2012. Our study was approved by Adnan Menderes University School of Medicine Ethical Committee.

The cases that myocardial pathology was detected in hematoxylin-eosin (HE) stained samples (AMI, former myocardial infarction, coronary atherosclerosis) were selected for immunohistochemical examinations. Selected cases were classified according to the presence of myocardial damage and coronary atherosclerosis stenosis degree. Cases were separated into 4 groups according to their causes of death: Group A included deaths from AMI by history, medical records and autopsy findings (in macroscopic and microscopic exam performed with HE stain) (n:19), Group B included the cases died because of a cardiac reason by clinical findings and macroscopic examination, but no AMI could be detected in the microscopic examination performed by using HE stain (n:7), Group C included the cases who died from noncardiac natural causes (n:8) and Group D included the cases who were reported to die as forced death (that is thought to have an impact on the apoptotic process) (n:9).

Immunohistochemical Method

Immunohistochemical staining was performed by using the Avidin-Biotin complex system (ABC).

Immunohistochemical bcl-2, bax and Fas stains were performed for the selected sections.

The sections to that bcl-2, bax and Fas were applied were examined, and positive staining in the myocardium was detected. Positive staining pattern for bcl-2, bax and Fas was evaluated cytoplasmically. It was evaluated as negative (−) if staining was observed in less than 10% of the myocardial cells and positive if staining was observed in more than 10% of the myocardial cells on the sections.

TUNEL Method

TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labelling) technique was used for the determination of apoptosis.

In cases that cells were marked with TUNEL method less than 10% were considered as negative, whereas the cases with cells more than 10% were considered positive.

Statistical Analysis

Statistical analysis was made with SPSS 17.0 for Windows (Statistical Programme for Social Sciences) program. The cases were evaluated sociodemographically, macroscopically and microscopically. In all assessments, p value <0.05 was accepted as significant with 5% error margin in 95% confidence interval.

RESULTS

Postmortem and immunohistochemical findings of all cases

In our study, 34 (79.1%) of the 43 cases were male and 9 (20.9%) were female. The youngest age was 19 whereas the oldest was 83 and the mean age was found as 54.49 ±16.37. Eight (18.6%) of the cases were above the age 40, 35 (%81.4) were below the age 40.

Thirty-six (83.7%) of the autopsies were performed in the first 24 hours following the death, four (9.3%) were between 25-48 hours, two (4.7%) in 49-72 hours and one (2.3%) was performed in more than three days. The origin of death was determined in 31 (62.1%) of the cases as natural causes as a result of a disease they already had, in seven (16.3%) cases as an accident, in five cases (11.6%) as suicide. As a result of the autopsies performed in our study, the reason of death was reported
as AMI in 19 (44.2%) cases, coronary artery disease in four (9.3%), and heart failure in two (4.7%) cases. Other deaths were due to noncardiac origins.

When the heart weights obtained from the autopsies were evaluated; the lightest heart was weighed as 219 g, the heaviest as 784 g and the mean weight of all the hearts as 460.9 g ±135.4 g. The mean heart weight in men was calculated as 480.1± 124.1 g whereas in women as 378.7±160.9 g. Cardiomegaly was evaluated as 300 g and more for males and 250 g and more for females.

No microscopically pathological feature was seen in 11 (25.6%) cases. The macroscopical features of the myocardiums of the cases were shown in details in Table 1.

AMI was detected in 19 (44.2%) cases, whereas in 24 (55.8%) cases AMI was not histologically seen. 13 (30.2%) of all the cases had previous MI findings, and atherosclerosis was detected in the aorta in 37 (86%) and in at least one coronary artery in 34 (79.1%) cases. There was stenosis more than 75% degree in the Left Anterior Descending (LAD) artery in seven (16.3%) cases, 50-74% degree stenosis in the LAD artery in seven (16.3%) cases, more than 75% degree stenosis in the Left Circumflex (LCX) artery in five (11.6%) cases, 50-74% degree stenosis in the LCX artery in four (9.3%) cases, more than 75% degree stenosis in the Right Circumflex Anterior (RCA) artery in four (9.3%) cases, 50-74% degree stenosis in the RCA artery in 2 (4.7%) cases.

No marking with TUNEL method was seen in 9 (20.9%) cases, whereas there was marking in 34 (79.1%) cases. When the cases that had cells that were marked with TUNEL method more than 10% were considered as positive, 18 (41.9%) cases were stained negative and 25 (58.1%) cases were stained positive. In the preparations that were stained positive with TUNEL method, the mean rate of the contrast stained apoptotic cells were 37.6±24.9%, whereas the least was 10% and the highest was 80%. The mean TUNEL staining rates of all cases were 28±26.7%, whereas the least was 1% and the highest was 80%.

There were 25 (58.1%) cases that stained positively with the TUNEL method. Out of 19 cases who were diagnosed with AMI, six (31.6%) were stained negatively with TUNEL and 13 (68.4%) were stained positively. There were no significant findings between the presence of AMI in the myocardium and staining with TUNEL (p=0.224). The comparison of the AMI cases regarding apoptotic immunohistochemical markers were shown in details in Table 2.

There were findings of previous myocardial infarction in 13 of them. There were no statistically significant correlation between previous myocardial infarction and apoptotic staining (p>0.005). The correlation of previous myocardial infarction with the staining of apoptotic markers is shown in Table 3. There was a significant correlation between the presence of 50% or more stenosis in any coronary artery and staining positively of the myocardium with bax (p=0.016) and Fas (p=0.008) (Table 4).

Postmortem and immunohistochemical findings of patient groups

The cases were divided into 4 groups and evaluated within themselves.

Group A: The findings of the cases that were diagnosed with AMI as the reason of death according to the history, medical records and autopsy findings (in the macroscopic and microscopically examination performed with HE stain) (n=19) are written below. The youngest case in Group A was 33 and oldest was 83. The mean age was 56.79±14.9. In Group A, 16

<table>
<thead>
<tr>
<th>Macrosopy</th>
<th>No pathological feature</th>
<th>Off-white necrosis area</th>
<th>Coronary artery with clogged lumen</th>
<th>Haemorrhagical area in the myocardium present</th>
<th>Necrotic area in myocardium and stenotic artery together</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Male</td>
<td>7 16.3</td>
<td>9 20.9</td>
<td>3 7</td>
<td>6 14</td>
<td>9 20.9</td>
<td>34 79.1</td>
</tr>
<tr>
<td>Female</td>
<td>4 9.3</td>
<td>2 4.7</td>
<td>0 0</td>
<td>1 2.3</td>
<td>2 4.7</td>
<td>9 20.9</td>
</tr>
<tr>
<td>Sum</td>
<td>11 25.6</td>
<td>11 25.6</td>
<td>3 7</td>
<td>7 16.3</td>
<td>11 25.6</td>
<td>43 100</td>
</tr>
</tbody>
</table>

Table 1. The macroscopical features of the myocardiums of the cases

<table>
<thead>
<tr>
<th>AMI (-)</th>
<th>AMI (+)</th>
<th>Sum</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>-</td>
<td>8 18.6</td>
<td>5 11.6</td>
</tr>
<tr>
<td>+</td>
<td>16 37.2</td>
<td>14 32.6</td>
<td>30 69.8</td>
</tr>
<tr>
<td>Bax</td>
<td>-</td>
<td>6 14</td>
<td>4 9.3</td>
</tr>
<tr>
<td>+</td>
<td>18 41.9</td>
<td>15 34.9</td>
<td>33 76.7</td>
</tr>
<tr>
<td>Fas</td>
<td>-</td>
<td>6 14</td>
<td>2 4.7</td>
</tr>
<tr>
<td>+</td>
<td>18 41.9</td>
<td>17 39.5</td>
<td>35 81.4</td>
</tr>
<tr>
<td>TUNEL</td>
<td>-</td>
<td>12 27.9</td>
<td>6 14</td>
</tr>
<tr>
<td>+</td>
<td>12 27.9</td>
<td>13 30.2</td>
<td>25 58.1</td>
</tr>
</tbody>
</table>

Table 2. The comparison of the cases with AMI and apoptotic immunohistochemical markers (the cases that were stained at any degree with bcl-2, bax and Fas and that had more than 10% of cells stained with TUNEL method were grouped as + and evaluated)
In the male cases in Group A, one’s (7.7%) heart weight was normal and in 12 (92.3%) of them the heart weight was calculated as increased. The heart weight was increased in all 3 female cases. In 17 (89.5%) of the cases, the time interval between the death and the autopsy was less than 24 hours and in two (10.5%) cases the autopsy was performed within 25-48 hours.

In the macroscopically examinations performed on the cases’ hearts, no pathological lesions were seen in three (15.8%) cases. There was off-white necrosis area in four (21.1%) cases, haemorrhagic area in two (10.5%) cases, coronary artery with clogged lumen in two (10.5%) cases.

Group B: The findings of the cases that the cause of death was thought to be a cardiac reason by the event, clinical findings and macroscopic examination of the heart and reported as a cardiac disease (coronary artery disease, myocarditis, heart failure), but no AMI could be detected in the microscopic examination performed by using HE stain (n:7) are written below.

There were six (85.7%) male and one female (14.3%) cases in Group B. In the second group, the youngest case was 47 and the oldest was 71 years old and the mean age was detected as 59.29±10.24. All the hearts’ weights were found to be increased in Group B except for one heart that was missing. In four (57.1%) of the cases the time interval between the death and the autopsy was less than 24 hours, in one (14.3%) case it was between 25-48 hours, in two (28.6%) cases it was between 49-72 hours.

In the macroscopically examinations performed on the cases’ hearts, no pathological lesions were seen in two (28.6%) cases. There was off-white necrosis area in one (14.3%) case, haemorrhagic area in one (14.3%) case, coronary artery with clogged lumen and off-white necrosis area together in two (28.6%) cases.

Group C: The findings of the cases that the certain cause of death was reported as noncardiac natural causes (encephalitis, acute pancreatitis, subarachnoidal bleeding, lung carcinoma, pulmonary haemorrhage) (n:8) are written below.

There were five (62.5%) male and three female (37.5%) cases in Group C. The youngest case was 24 and the oldest was 75 years old and the mean age was detected as 56.13±16.1. Seven (87.5%) of the cases were 40 years old and above. In six (75%) of the cases the time interval between the death and the autopsy was less than 24 hours, in one (12.5%) case it was between 25-48 hours, in one (12.5%) case it was performed in less than 3 days. All the heart’s weights were found to be increased in the third group except for one heart that was missing.

In the macroscopically examinations performed on the cases’ hearts, no pathological lesions were seen in three (37.5%) cases. There was off-white necrosis area in three (37.5%) cases, haemorrhagic area in one

---

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Previous MI (-)</th>
<th>Previous MI (+)</th>
<th>Sum</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Bcl-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>9</td>
<td>20.9</td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td>+</td>
<td>21</td>
<td>48.8</td>
<td>9</td>
<td>20.9</td>
</tr>
<tr>
<td>Bax</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>8</td>
<td>18.6</td>
<td>2</td>
<td>4.7</td>
</tr>
<tr>
<td>+</td>
<td>22</td>
<td>51.2</td>
<td>11</td>
<td>25.6</td>
</tr>
<tr>
<td>Fas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>7</td>
<td>16.3</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>+</td>
<td>23</td>
<td>53.5</td>
<td>12</td>
<td>27.9</td>
</tr>
<tr>
<td>TUNEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>14</td>
<td>32.6</td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td>+</td>
<td>16</td>
<td>37.2</td>
<td>9</td>
<td>20.9</td>
</tr>
</tbody>
</table>

---

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Coronary atherosclerosis (-)</th>
<th>Coronary atherosclerosis (+)</th>
<th>P</th>
<th>Coronary stenosis less than 75%</th>
<th>75% and more coronary stenosis</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>4.7</td>
<td>11</td>
<td>25.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>7</td>
<td>16.3</td>
<td>23</td>
<td>53.5</td>
<td>.699</td>
<td></td>
</tr>
<tr>
<td>Bax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>16.3</td>
<td>.413</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>6</td>
<td>14</td>
<td>27</td>
<td>62.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>11.6</td>
<td>.332</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>6</td>
<td>14</td>
<td>29</td>
<td>67.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUNEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>9.3</td>
<td>14</td>
<td>32.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>5</td>
<td>11.6</td>
<td>20</td>
<td>46.5</td>
<td>.824</td>
<td></td>
</tr>
</tbody>
</table>

---

(84.2%) cases were male and 3 (15.8%) cases were female. In the male cases in Group A, one’s (7.7%) heart weight was normal and in 12 (92.3%) of them the heart weight was calculated as increased. The heart weight was increased in all 3 female cases. In 17 (89.5%) of the cases, the time interval between the death and the autopsy was less than 24 hours and in two (10.5%) cases the autopsy was performed within 25-48 hours.

In the macroscopically examinations performed on the cases’ hearts, no pathological lesions were seen in three (15.8%) cases. There was off-white necrosis area in four (21.1%) cases, haemorrhagic area in two (10.5%) cases, coronary artery with clogged lumen in two (10.5%) cases.

Group B: The findings of the cases that the cause of death was thought to be a cardiac reason by the event, clinical findings and macroscopic examination of the heart and reported as a cardiac disease (coronary artery disease, myocarditis, heart failure), but no AMI could be detected in the microscopic examination performed by using HE stain (n:7) are written below.

There were six (85.7%) male and one female (14.3%) cases in Group B. In the second group, the youngest case was 47 and the oldest was 71 years old and the mean age was detected as 59.29±10.24. All the hearts’ weights were found to be increased in Group B except for one heart that was missing. In four (57.1%) of the cases the time interval between the death and the autopsy was less than 24 hours, in one (14.3%) case it was between 25-48 hours, in two (28.6%) cases it was between 49-72 hours.

In the macroscopically examinations performed on the cases’ hearts, no pathological lesions were seen in two (28.6%) cases. There was off-white necrosis area in one (14.3%) case, haemorrhagic area in one (14.3%) case, coronary artery with clogged lumen and off-white necrosis area together in two (28.6%) cases.

Group C: The findings of the cases that the certain cause of death was reported as noncardiac natural causes (encephalitis, acute pancreatitis, subarachnoidal bleeding, lung carcinoma, pulmonary haemorrhage) (n:8) are written below.

There were five (62.5%) male and three female (37.5%) cases in Group C. The youngest case was 24 and the oldest was 75 years old and the mean age was detected as 56.13±16.1. Seven (87.5%) of the cases were 40 years old and above. In six (75%) of the cases the time interval between the death and the autopsy was less than 24 hours, in one (12.5%) case it was between 25-48 hours, in one (12.5%) case it was performed in less than 3 days. All the heart’s weights were found to be increased in the third group except for one heart that was missing.

In the macroscopically examinations performed on the cases’ hearts, no pathological lesions were seen in three (37.5%) cases. There was off-white necrosis area in three (37.5%) cases, haemorrhagic area in one
In a study by Edston et al. [30], it was stated that in the study group that was diagnosed with coronary artery disease and myocardial infarction, apoptosis was seen in all sections with the TUNEL method, the mean percentages of apoptotic myocyte nuclei were 26% in the vicinity of infarction (MI) and 32% in the myocardium far away from the infarct and in the right ventricular myocardium, and in the group of sudden cardiac deaths without CAD, apoptosis was seen in all sections with the TUNEL method, but scarcely within necrotic lesions where the cellular nuclei were either absent or severely disintegrated, diffuse TUNEL positivity was seen in both the left (mean values 25 and 25%) and right heart and when the apoptosis rates of both groups that had cardiac pathologies were compared to the control group, it was found statistically significant.

In a study by Rodriguez-Calvo et al. [31], it was stated that in the group that had a histological diagnosis of myocardial infarction, the percentage of stained nuclei ranged from 0% to 74.7% (average, 36%), in the group that had possible ischemic originated sudden death cases without the histological evidence of myocardial injury, the percentage of positive nuclei ranged from 27% to 47% (average, 32.5%), in the cases that had ischemic damage without last phase ischemic lesions the percentage of positive nuclei ranged from 0% to 43% (average, 20.3%), in the group of sudden deaths caused by a reason other than a cardiological disease, unaffected, histological normal cardiomyocytes exhibited negative nuclear and cytoplasm staining.

In literature, it is stated that the apoptotic index varies quite a lot and that it is thought to depend on the subjectivity of different observers that evaluate the results of TUNEL staining. In a study by Piro et al. [5], it was stated that immunohistochemical stains were always negative in contrast with other results that showed an increase in bcl-2 and Fas. However, in most of these studies, the hearts came from experimental animals or from autopsies performed within 6 hours of the patient's death, Piro et al. [5] stated that their autopsy myocardial samples were all taken 24 hours after death. There was no significant correlation between the group that the cause of death was AMI and myocardial injury and TUNEL staining in our study.

Apoptosis studies in ischemic hearts show a lot of differences [5, 32, 17, 21, 33-39] and this brings up the questions about the sensitivity and specificity of the TUNEL method. Experiences show that the TUNEL method may lead to false positive and false negative results and this can be explained by staining kinetics [40]. However, postmortem autolysis has not been shown to critically effect the staining at least for the first two days [41]. It is said that the active RNA synthesis [42] and DNA damage [40, 43] in necrotic cells may cause nonspecific staining. In order to obtain reliable and productive results, the
TUNEL method may carefully be standardized by staining of a tissue that contain multiple apoptotic cells and a tissue that has no apoptosis such as myocardium.

An unexpected finding such as the presence of apoptotic nuclei in non-ischemic areas may be interpreted as the result of the mechanical stress in the ventricular myocytes due to the increased parietal stress and loading [44].

Misao et al. [24] reported that bcl-2 protein is secreted in the saved myocytes of the acute infarcted human hearts; this situation gives rise to the thought that some heart cells may be protected by the expression of bcl-2 in early phases of infarction. Even though bcl-2 protein expression was seen in the saved myocytes that surround infarcted tissues, it is reported that there was a significant increase in the secretion of bax paraapoptotic protein in the infarcted area [45].

In the study with rat ischemia-reperfusion model by Celkan et al. [46], it was stated that there was a significant decrease in the rate of apoptotic cells in the infarcted area with the bcl-2 immunohistochemical staining method in the group that had single or repeated short term ischemia periods compared to the group that was exposed to ischemia for a long time and there was a significant difference between the non-ischemic control group and the other two groups in terms of apoptotic cells.

In a study by Olivetti et al. [47], it was stated that bcl-2 protein was seen more in the myocyte cytoplasm and bcl-2 positive cells were seen more in damaged myocytes, bax protein had a similar cytoplasmic localization but it did not make a difference between the control group and the damaged myocytes. The correlation between the presence of atherosclerosis in the coronary arteries of the cases and the apoptotic markers was not statistically significant (p>0.005).

The reason for not getting significant findings might be the unknown things such as the time of exposure to ischemia of the cases in our study, the reperfusion status of the process before death and the shortcomings such as the lack of the number of the cases.

There were necrotic findings of previous myocardial infarction in 13 cases. There was no significant correlation between having a myocardial infarction and apoptotic staining (p>0.005). Even though the cardiac decompensation mechanism is not all clear, it most likely includes progressive contraction dysfunction and/or progressive cardiomyocyte degeneration and death.

Heart weight differs according to the age and gender. In their study, Bardeles et al. accepted the normal heart weight as 250-300 g in females and 300-350 g in males and in 22 cases out of 35 in the case group that carry high risk for MI without early histological findings of MI had cardiomegaly and in the group that has 13 cases with histological normal myocardium, two of the cases had normal heart weight [33]. In the study by Arnold et al., in the cases that died of a chronic disease they already had, the heart weight average was 253 g in females and 284 g in males [48]. The reason why this much cardiomegaly is detected may be that the majority of the cases that were involved in the study had cardiac and chronic diseases.

Inflammatory heart disease shows different clinical findings and symptoms that range from loss of function in different intensity to chronic severe heart failure that causes fulminant myocarditis [49]. Apoptosis is known to cause cardiomyocyte loss in inflammatory heart disease [47, 49-59].

In the study by Kytö et al. [60], it was stated that analysis of apoptosis demonstrated TUNEL positive stained cardiomyocytes in the hearts of all cases, per patient, an average of 2.0-0.3% of the cardiomyocytes showed apoptotic DNA fragmentation and very few apoptotic cardiomyocytes were found in the controls, namely 0.008 - 0.003% by TUNEL assay. There was no statistically significant correlation between myocarditis and apoptosis and the amount of apoptosis did not correlate with the age or gender of the cases, time from death to autopsy, assumed viral aetiology of myocarditis, histopathology features, severity of myocarditis, or duration of clinical disease. In the study by Alter et al. [61], it was stated that the percentages of TUNEL-positive stained cardiomyocytes were increased (P<0.05) in endomyocardial biopsies from patients with chronic myocarditis when compared with controls. There was one case diagnosed with myocarditis in our study. In the immunohistochemical examination of this case with the TUNEL method, 30% of the myocardial cells were stained positively and there was moderate (++) staining with bcl-2, bax and Fas. Even though it is not possible to correlate myocarditis and apoptosis statistically over one case, our study can be interpreted as supportive of the other cases that we have mentioned above.

CONCLUSIONS

The use of apoptosis in the myocardial tissue of the cases that died of AMI for postmortem early diagnosis is thought to be helpful. Bcl-2, bax and Fas markers and the TUNEL staining method are thought to be used in showing positivity in these cases. Although the TUNEL staining is sensitive in determination of early MI, we think that its use may be insufficient due to its low specificity. It may be useful to support this method with others.

In our study, in the cases that were diagnosed as AMI and CAD with possible AMI, the apoptosis markers and TUNEL were stained more positively.

In the diagnosis of sudden unexpected deaths, while detecting the cause of death and mechanism, there are many challenges that are faced with. For the
early phase diagnosis of AMI, a lot of biochemical, histochemical, immunohistochemical methods are being researched. In order to help decreasing the challenges and accelerating the justice, evaluation of cardiovascular system in especially sudden unexpected deaths should be performed by learning the medical history, judicial investigation, crime scene investigation, laboratory investigation, autopsy, histopathological investigation at the earliest possible time.

References