นิพนธ์ต้นฉบับ

DOI : 10.14456/clmj.2018.7 — Chula Med J Vol. 62 No. 4 July - August 2018

Demonstration of myocardial infarction in decomposed myocardium with vascular endothelial growth factor immunohistochemistry: A tropical climate study

Parath Thirati*

Panuwat Chutivongse*

Thirati P, Chutivongse P. Demonstration of myocardial infarction in decomposed myocardium with vascular endothelial growth factor immunohistochemistry: A tropical climate study. Chula Med J 2018 Jul – Aug;62(4): 711 - 23

Background : *Tissue damage caused by decomposition contributes to difficulties faced by forensic pathologists in medico-legal autopsy. Various studies have utilized immunohistochemistry in decomposed forensic caseworks, including myocardial infarction (MI). To date, only few markers have been studied in decomposed MI specimens. Moreover, there are no researches that performed in tropical climate areas. This study is the first study to perform vascular endothelial growth factor (VEGF) immunohistochemistry in decomposed MI samples. This is also the first paper on performed immunohistochemistry in tropical climate areas.*

Objective : *To study whether VEGF immunohistochemistry can be used in decomposed MI specimens in tropical climate areas. Secondary objective is the longest decomposition period that it could be used if the primary objective is possible.*

Methods : *MI and non-MI specimens from medico-legal autopsy cases were sampled and stored for 0, 1, 2, 3, and 5 days. When the storage times for each specimen were reached, the tissues were then processed and stained by haematoxylin and eosin (H&E) staining, myoglobin (only in fresh specimens), and VEGF immunohistochemistry.*

*Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University

Results : *Comparing VEGF immunohistochemistry staining between MI and non-MI groups, there were statistically significant difference of staining between the groups from fresh specimen up to decomposition period of 2 days. Comparing stainability of VEGF among specimens at different decomposition periods with fresh specimens, there was no statistically difference between fresh specimens and specimens with decomposition periods of 1 and 2 days.*

Conclusion : *In tropical climate, VEGF immunohistochemistry can detect MI until decomposition periods of 2 days. However, in early MI specimens, VEGF may still detect MI at decomposition period of 5 days.*

Keywords : *Decomposition, myocardial infarction, vascular endothelial growth factor, immunohistochemistry.*

Correspondence to: Chutivongse P, Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Received for publication. January 19, 2018.

ปรัตถ์ ถิราติ, ภาณุวัฒน์ ชุติวงศ์. การตรวจหากล้ามเนื้อหัวใจขาดเลือดในกล้ามเนื้อหัวใจเน่าด้วยการย้อม vascular endothelial growth factor โดยวิธีอิมมูโนพยาธิวิทยา: การศึกษาในภูมิอากาศ เขตร้อน. จุฬาลงกรณ์เวชสาร 2561 ก.ค. – ส.ค.;62(4): 711 – 23

เหตุผลของการทำวิจัย : *การเน่าเป็นอุปสรรคที่สำคัญของการผ่าชันสูตรศพทางนิติเวชศาสตร์ มีการศึกษาในต่างประเทศที่ใช้การย้อมตรวจด้วยวิธีอิมมูโนพยาธิวิทยามาใช้ในการวินิจฉัยพยาธิสภาพในศพเน่า รวมทั้งกรณีกล้ามเนื้อหัวใจขาดเลือด อย่างไรก็ตามในตัวอย่างกล้ามเนื้อหัวใจขาดเลือดที่เน่า การศึกษายังคงจำกัดอยู่ในชนิดการย้อมด้วยวิธีการทางอิมมูโนพยาธิวิทยาเพียงไม่กี่ชนิด นอกจากนี้ยังไม่มีการศึกษาการใช้วิธีอิมมูโนพยาธิวิทยาในตัวอย่างที่เน่าในภูมิอากาศเขตร้อน งานศึกษานี้เป็นงานศึกษาแรกที่ศึกษาการย้อม vascular endothelial growth factor (VEGF) ในกล้ามเนื้อหัวใจเน่าและเป็นการศึกษาแรกที่ใช้วิธีอิมมูโนพยาธิวิทยาในศพเน่าในสภาพอากาศเขตร้อน*

วัตถุประสงค์ : *ศึกษาความเป็นไปได้ของการย้อม vascular endothelial growth factor (VEGF) โดยวิธีอิมมูโนพยาธิวิทยาเพื่อตรวจหากล้ามเนื้อหัวใจขาดเลือดในตัวอย่างที่เน่า และระยะเวลาการเน่าที่นานที่สุดที่จะตรวจได้หากสามารถตรวจย้อมได้*

วิธีการทำวิจัย : *เก็บตัวอย่างกล้ามเนื้อหัวใจจากศพที่ผ่านการผ่าชันสูตรทางนิติเวชศาสตร์โดยแบ่งกลุ่มออกเป็นกลุ่มที่เสียชีวิตจากกล้ามเนื้อหัวใจขาดเลือดและกลุ่มที่เสียชีวิตจากสาเหตุอื่น โดยเก็บตัวอย่างไว้ 0,1, 2, 3 และ 5 วัน จากนั้นนำชิ้นเนื้อไปผ่านกระบวนการและตรวจย้อมด้วย Haematoxylin & Eosin การตรวจย้อมด้วย myoglobin (เฉพาะตัวอย่างที่เก็บไว้ 0 วัน) และ VEGF*

ผลการศึกษา : *พบความแตกต่างอย่างมีนัยสำคัญทางสถิติโดยการย้อม VEGF ระหว่างกลุ่มที่เสียชีวิตจากกล้ามเนื้อหัวใจขาดเลือด และกลุ่มที่เสียชีวิตจากสาเหตุอื่น ในชิ้นเนื้อสดและชิ้นเนื้อที่เก็บไว้ให้เน่า 1 และ 2 วัน การเปรียบเทียบการย้อม VEGF ภายในกลุ่มที่เสียชีวิตจากกล้ามเนื้อหัวใจขาดเลือด ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกล้ามเนื้อหัวใจสดและกล้ามเนื้อหัวใจ ที่เก็บไว้ให้เน่า 1 และ 2 วัน*

สรุป : *ในภูมิอากาศเขตร้อน สามารถใช้การตรวจหา VEGF โดยวิธีอิมมูโนพยาธิวิทยาได้จนถึงการเน่าวันที่ 2 อย่างไรก็ตามในตัวอย่างกล้ามเนื้อหัวใจขาดเลือดระยะแรก ยังสามารถตรวจได้หลังจากการเน่าวันที่ 5*

คำสำคัญ : *การเน่า, กล้ามเนื้อหัวใจขาดเลือด, vascular endothelial growth factor, อิมมูโนพยาธิวิทยา.*

Decomposition is one of the most difficult cases faced by forensic pathologists. Apart from unsatisfactory conditions of the body and limited of information about the circumstance of death, partly due to social isolation [1], decomposition itself also wreak havoc on much of the body tissues. As a result, pathological lesions could hardly be examined, and toxicological specimen could barely be collected for laboratory analysis.[1,2] Hence, forensic pathologists could hardly make the diagnosis after performing an autopsy.

Immunohistochemistry is a pathological technique that is widely accepted and used in both anatomical and forensic pathology caseworks. Multiple studies had performed immunohistochemistry in decomposed forensic specimens including anaphylaxis[3], contusions[4, 5], Alzhemer's disease[6, 7], and also, myocardial infarction (MI).[8, 9] Unfortunately, these publications have been performed in temperate climateareas. As temperature is one of the most important factors that affect decomposition process[2, 10], there is a need to verify whether immunohistochemistry could be utilized in decomposed forensic pathology caseworks in tropical climate areas, including Thailand. To date, no studies have explored these possibilities.

Ischemic heart disease is the most common cause of death in sudden unexpected death of the adults worldwide.[11] Although diagnosis of MI by immunohistochemistry has been extensively studied and it is still an ongoing research topic in forensic pathology [12 - 14], detecting MI in decomposed specimens is still a challenging issue. In decomposed cases, apart from the atherosclerotic calcification left in coronary vessels or fibrotic scar of myocardial tissue, myocardium could hardly be examined by routine gross and microscopic examination. [2,15] MI could only be diagnosed when special pathological techniques, including immunohistochemistry, are employed. Ortmann C *et al*. and Thomsen H *et al*. have showed that demonstration of myocardial infarction lesions is possible in advanced decomposed specimens. However, only few markers were studied in their publications.[8,9] Moreover, as previously stated, no information regarding caseworks in tropical climate areas is available until now.

The goal of this study is to explore the possibilities of detecting MI lesions by vascular endothelial growth factor (VEGF) immunohistochemistry in decomposed specimens in tropical climate areas. Moreover, if it is possible, the longest period it could be detected after decomposition sets in. As VEGF have been showed to be positive in MI tissues[16,17] and can also be performed in various anatomical pathology laboratories in Thailand. The authors decided to utilized VEGF as the studied immunohistochemistry markers to answer the stated research questions.

Material and Methods

Ethical considerations

This study was approved by the Institution Review Board, Faculty of Medicine, Chulalongkorn University (IRB No.668/59; COA No.046/2017) in compliance with the international guidelines for human research protection as in the Declaration of Helsinki, the Belmont Report, CIOMS guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Case selection

All the myocardium specimens were obtained from the cases that were determined necessary for medico-legal autopsy according to the Thai Criminal Procedure Code by forensic pathologists and inquiry police officers after crime scene investigation. All the cases were autopsied at the Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University from March 2017 to June 2017. Ten cases were selected for the MI group and five cases were selected for the control group.

Inclusion criteria for the MI groups were: (1) Cause of death (COD) was certified as MI with visible gross pathology. (2) Microscopic findings of the fresh specimens were consistent with MI by haematoxylin and eosin (H&E) staining and immunohistochemistry staining with myoglobin and vascular endothelial growth factor (VEGF). (3) Subject was at least 20 years-old.

Exclusion criteria for the MI group were: (1) History of blunt force trauma, including cardiopulmonary resuscitation (CPR), at the chest occurred at the time of death (2) Postmortem Interval (PMI) at mortuary arrival was more than 18 hours. (3) Signs of decomposition were observed at the time of autopsy.

Inclusion criteria for the control group were: (1) COD was not certified as MI or ischemic heart disease. (2) MI was not detected by microscopic examination with haematoxylin and eosin (H&E) staining and immunohistochemistry staining with myoglobin and vascular endothelial growth factor (VEGF). (3) Subject was at least 20 years-old.

Exclusion criteria for the controls were: (1) The same exclusion criteria of the MI group were also applied. (2) At least 20 percent of coronary occlusions in at least one coronary artery was detected at the autopsy. (3) Evidence of MI was observed at the autopsy. (4) Evidence of previous coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI) was presented.

Autopsy and specimen collection

Autopsies were performed as early as possible. If the body arrived at the mortuary after working hours, it would be stored in a body refrigerator (at -2 to 5 $^{\circ}$C) until autopsy was performed in the next morning (maximum storage time until autopsy is 20 hours). Informed consent by the next of kin was obtained in all of the cases in this study. Autopsy and necessary toxicological investigations were performed in accordance with the routine standard protocol. In each case, 5 pieces of myocardium were sampled and cut into 5 pieces of quadrangles, measuring approximately 1.0 centimeters in length and width and approximately 0.3 centimeter in thickness. In MI groups, myocardium was cut to include both infarction zone and normal zone. Each piece of specimens was placed in a plastic tissue cassette and then was stored in a concealed plastic container at room temperature.

In each case, one piece of the samples was immediately stored in 10% neutral buffer formalin (Bio-Optica; Italy), and was designated as Decomposition Day (DD) 0 (fresh specimen). After an overnight incubation, it was then processed and fixed in a paraffin block before being further stained.

Since postmortem intervals of decomposed cases faced by forensic pathologists at our institution mostly fall between 1 to 5 days, we decided to set the maximum period of decomposition in this study at 5 days. Hence, the second, the third, the fourth, and the fifth pieces of myocardium tissues were stored at room temperature for 1 day, 2 days, 3 days, and 5 days and were labelled as Decomposition Day (DD) 1, DD 2, DD 3, and DD 5 consecutively. After storage time of each specimen was reached, the tissues were then stored and processed in the same way with the first piece of specimen (DD 0; fresh specimen).

Staining method

H&E staining was performed according to the routine standard protocol. Myoglobin was only performed on fresh specimen (DD 0). VEGF was performed on all pieces of specimens. Samples in paraffin blocks were sectioned for immunohistochemistry staining on positively charged microscopic glass slides (Superfrost TM Plus; Thermo Scientific) on the Leica Microsystems Bond Max System (Leica Microsystems; Bannockburn, IL). Slides were incubated for 60 minutes at 60°C and treated with Bond Dewax Solution (Leica Microsystems). Epitope retrieval was performed by incubating slides in Bond Epitope Retrieval Solution 2 (Leica Microsystems) for 30 minutes at 100°C. Immunohistochemistry was performed by a 3-step indirect immunoperoxidase technique using the Bond Polymer Refine Detection kit (Leica Microsystems). Primary antibody was applied for 45 minutes at room temperature followed by 3 consecutive rinses with Bond Wash Solution. Antibody for VEGF was monoclonal mouse anti-Human VEGF (Clone VG1, Code No. M7273; DAKO) at dilution 1: 200 with placenta as positive control. Antibody for myoglobin was polyclonal rabbit anti-human myoglobin (Code No. A324; DAKO) at dilution 1:10000 with skeletal muscle as positive control. Post Primary Polymer (Leica Microsystems) was applied for 8 minutes before rinsing 3 times with Bond Wash Solution. Polymer Poly-HRP IgG (Leica Microsystems) was applied for 8 minutes and rinsed 3 times with Bind Wash Solution and once with deionized water before diaminobenzidine chromogen was applied for 4 minutes followed by 3 times of deionized water rinsing. Slides were counterstained with hematoxylin for 5 minutes.

Microscopic examination

H&E staining slides in each case were examined for evidence of MI, stages of infarction and changes caused by decomposition. VEGF staining was qualitatively graded and recorded in the same way as Sabattaso S, *et al.* 2016 0 = negative or non-specific background staining, + = staining of single cells, ++ = staining of cell groups, and +++ = large/diffused staining.[13]

Statistical analysis

VEGF staining of tissues between the MI and control groups at the same DDs were compared with Mann-Whitney U test. VEGF staining of tissues in the MI group at different Decomposition Days (DD) and fresh specimen (DD 0) were compared by Friedman Test with Wilcoxson- signed rank test was further employed as post-hoc analysis. P - value < 0.05 was considered as statistically significant. IBM SPSS Version 22 was used as the statistical analysis program.

Results

Samples

The demographic data, postmortem interval at the time of crime scene investigation, transportation time, storage time, stages of MI were as detailed in Table 1 for the MI group and Table 2 for the control group.

Microscopic finding at different days of decomposition

Microscopic finding of tissues at different DDs were as illustrated in Figure 1. In general, at DD 1 tissues were still almost as the same as fresh specimens. Bacterial colonization appeared at the margins of the specimen. At DD 2, bacterial colonization was increased and denser than what was

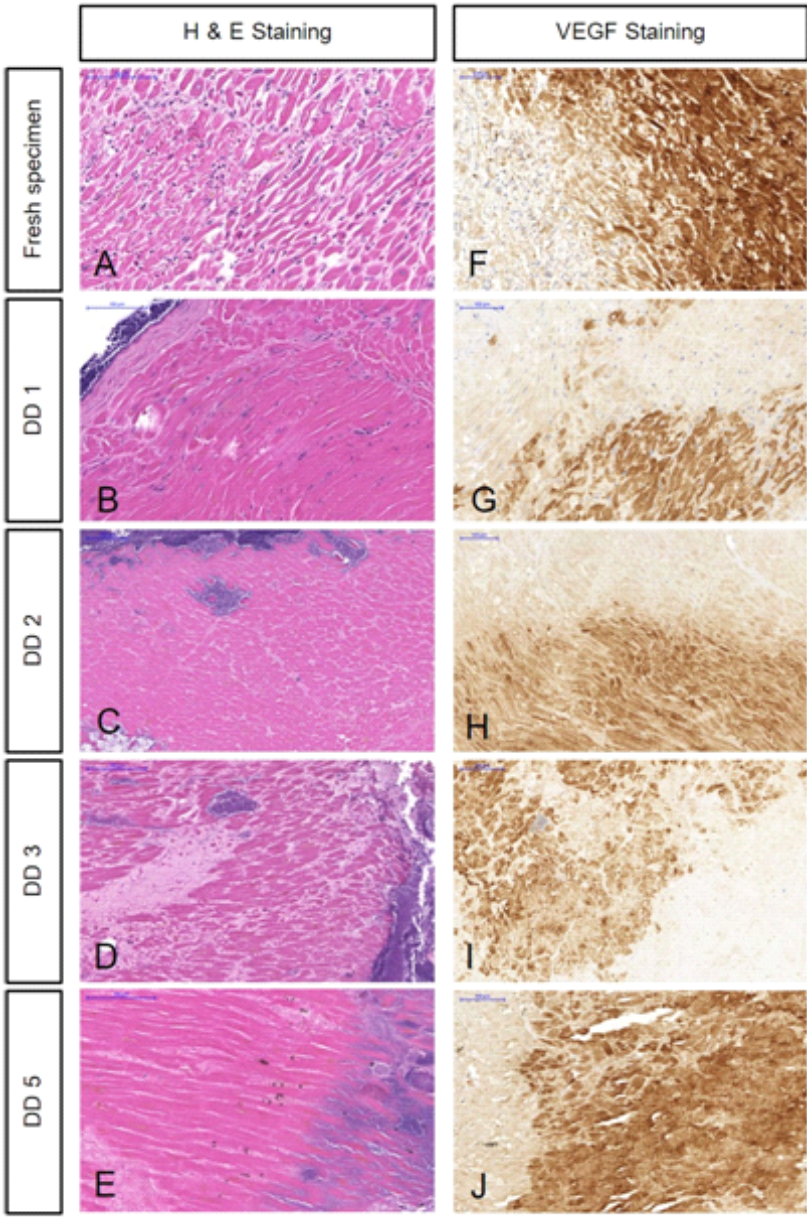

Figure 1. (MI Case No.1) The degree of tissue destruction and the number of bacterial colonies progressed as decomposition period increased. (A to E) In early MI cases, VEGF staining was strongly positive in fresh specimen and was also strongly positive despite advanced decomposition (F to J).

found at DD 1. Architecture of the tissues were still intact but details of the myocytes, including nuclei and striations began to be disappeared. Also, leucocytes and red blood cells could not also be differentiated. At DD 3, myocytes began to shrink but the overall architecture of the tissue samples were still intact. Bacterial colonization was found in multiple areas of the tissue. At DD 5, the increased degree of myocardial shrinkage was observed with intact connective tissue. Bacterial clumps were also increased.

Immunohistochemistry

Myoglobin staining could demonstrate areas of negative cytoplasmic staining in all cases in the MI group, confirming the present of MI (Figure 2). While in the control group, no area of negative staining could be detected. VEGF was detected at the cytoplasm of myocytes at peri-infarction areas (border zones of infarction areas) (Figure 1). The details of VEGF staining in each case were depicted in Table 1 for the MI group and Table 2 for the control group. Differences of VEGF stainability between the two groups by Mann-Whitney U test were statistically significant at fresh specimen (DD 0), DD 1, and DD 2 (P = 0.002, 0.002, and 0.018 respectively); however, the difference of VEGF staining at DD 3 and DD 5 could not be detected (P = 0.115 and 0.299 respectively). Freidman test could detect statistical difference of VEGF staining among the MI group at different DDs with P <0.001. Post hoc analysis by Wilcoxson- signed rank test, comparing VEGF staining of DD 0 with other different DDs in the group showed that at DD 1 and DD 2, there were no statistically different of VEGF staining from at DD 0 (P = 1.000 and 0.083 respectively). However, comparing VEGF staining between at DD 0 with staining of DD 3 and DD 5 showed statistically different staining (P = 0.009 and 0.011 respectively).

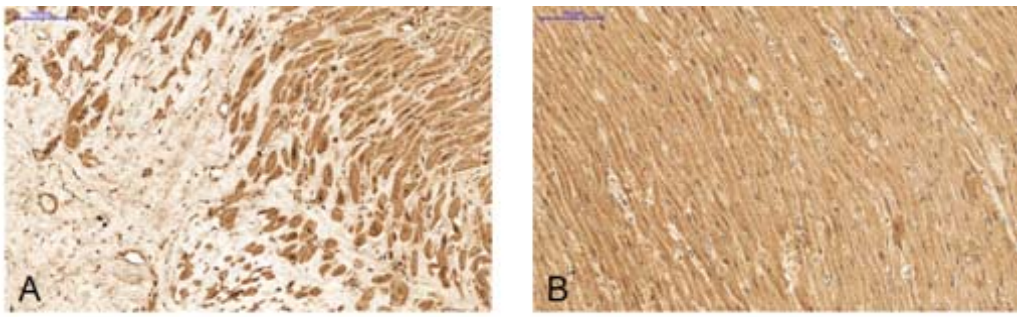

Figure 2. Confirmation of myocardial infarction by myoglobin staining. **A.** Depletion of myoglobin staining in MI tissue while strong myoglobin staining was observed in normal zone. (MI Case No.2) **B.** No depletion was observed in non-infarct myocardium. (Control Case No.4)

Table 1. Details of the MI group.

Case No.	Sex	Age (yr.)	PMI at Scene (hr.)	Time to Mortuary (hr.)	Storage Time (hr.)	Heart Weight (g.)	Occluded Vessels	Infarcted Area	Stage of Infarction	VEGF Day 0	VEGF Day 1	VEGF Day 2	VEGF Day 3	VEGF Day 5
1	M	57	8 - 12	2	10	390	LAD	Ant	Coagulation necrosis with brisk neutrophil infiltration	+++	+++	+++	+++	+++
2	M	73	1 - 2	1	4	425	LAD, LCX, RCA	Cir	Late coagulation necrosis	++	++	++	0	0
3	M	69	1 - 2	6	8	480	LAD	Ant, Sep	Early granulation tissue	+	+	0	0	0
4	F	46	1 - 2	6	11	450	LAD	Ant, Sep	Coagulation necrosis with few neutrophil infiltration	++	++	++	0	0
5	M	57	1 - 2	1	1	430	LCX, RCA	Lat, Pos	Late granulation tissue	+	+	0	0	0
6	M	53	2 - 3	4	11	450	LAD, RCA	Pos	Coagulation necrosis with brisk neutrophil infiltration	+++	+++	+++	++	0
7	F	61	4 - 6	4	0	410	LAD	Ant	Coagulation necrosis with brisk neutrophil infiltration	+++	+++	+++	+++	+++
8	M	58	4 - 6	3	17	465	LAD	Sep	Early granulation tissue	+	+	0	0	0
9	M	55	4 - 6	2	10	400	LAD	Ant	Coagulation necrosis with brisk neutrophil infiltration	+++	+++	+++	++	0
10	M	50	8 - 12	2	4	400	LAD	Ant	Coagulation necrosis with few neutrophil infiltration	++	++	++	0	0

PMI = Post mortem interval, LAD = Left anterior descending coronary artery, LCX = Left circumflex coronary artery, RCA = Right coronary artery, Cir = Circumferential, Ant = Anterior wall, Lat= Left ventricular free wall, Pos = Posterior wall

Table 2. Details of the control group.

Case No.	Sex	Age (yr.)	Cause of death	PMI at scene (hr.)	Time to mortuary (hr.)	Storage time (hr.)	Heart weight (g.)	VEGF Day 0	VEGF Day 1	VEGF Day 2	VEGF Day 3	VEGF Day 5
1	F	21	Hanging	8 - 12	2	10	275	0	0	0	0	0
2	M	19	Hanging	1 - 2	1	4	300	0	0	0	0	0
3	M	34	GI Bleeding	1 - 2	6	8	325	0	0	0	0	0
4	F	46	Hanging	1 - 2	6	11	250	0	0	0	0	0
5	M	40	Gunshot Wound	1 - 2	1	1	350	0	0	0	0	0

Discussion

VEGF is a signaling peptide that promotes vasculogenesis and angiogenesis, both of which are crucial for many physiological and pathological processes; for instance, fetal development, tissue repair, wound healing, tumor proliferation, necrotizing enterocolitis, rheumatoid arthritis, macular degeneration, etc. Abundant researches have studied the roles of VEGF in pathological processes together with therapeutic possibilities in various diseases.[18]

VEGF expression is triggered by several factors such as hypoxia, mechanical force, endotoxin etc. To date, it is accepted that hypoxia is the most important factor for VEGF expression. When tissue hypoxia occurs, hypoxia-inducible factor 1 (HIF-1) binds to hypoxia response element (HRE) which then triggering the expression of VEGF and several other proangiogenic growth factors.[18 - 20] In tissue restoration process, including MI, angiogenesis and vasculogenesis of VEGF expression occurred mostly at the parenchymal cells at the border of injured tissues and have paracrine effects on endothelial cells. In MI, the roles of VEGF are to promote endothelial cell migration, survival, proliferation, and development.[20, 21] Moreover, Messadi E, *et al.* also suggested that VEGF also improved mitochondrial respiratory function in ischemic- reperfusion injury in acute MI.[22] The overall results of VEGF expression after MI increased myocytes survival, reduced infarct size, and improved cardiac function. [20 - 22]

The result of this study is correspondence with previous studies on VEGF expression in MI. VEGF expression starts within the first 24 - 48 hours after MI.[16, 17, 23 - 25] Xu XH, *et al.* and Zhu BL, *et al.* found that VEGF immunohistochemistry were strongly positive in myocytes of the border zone of infarction in early phases of MI. [16 - 17] In this study, the same result was observed in the early MI cases of the MI group (Case 1, 6, 7 and 9). On the other hand, VEGF was weakly stained in the later phases of MI (Case 3, 5, and 8).

Several studies have explored decreased expression of VEGF in later stages of MI, however, no consensus has been established on this issue. Siddiqui AJ, *et al.* suggested that decreased expression of VEGF in subacute stages of MI might be related to decreased myocyte contraction because of MI.[23] Zhao T, *et al.* hypothesized that VEGF might be crucial only in initial stages of post-MI angiogenesis but not in later phases. Hence, other growth factors are upregulated while VEGF expression is suppressed in

the late stages of post-MI angiogenesis.[24] Recent studies have showed that local copper deficiency is presented after MI. As copper is among important elements in the transcription of VEGF and several other HIF-1 dependent proangiogenic growth factors, decreased expression of VEGF is due to copper deficiency secondary to MI. [25, 26]

Considering the result of VEGF staining in this study, VEGF persistence could be detected in advanced stages of decomposition in strongly positive cases; however, stainability began to be negative from DD 2 onward in some cases. It is possible that the amount of VEGF on the myocytes was gradually decreasing with time. Since the initial amount of VEGF in strongly positive cases was abundant, the decreased amount of VEGF on myocytes at DD 5 was still higher than the detection threshold of the performed immunohistochemistry. On the other hand, the initial amount of VEGF presented in weakly positive cases was low, as decomposition progressed, the number of VEGF was deteriorating until it started to be lower than the detection threshold at DD 2. Increased dilution of VEGF- antibody might also improve the study outcome. None the less, it might be assumed that in appropriately specimens (i.e. decomposed specimens of early phases of MI), VEGF could detect myocardial infarction even in advanced stages of decomposition (as seen in case 1 and 7).

Although the result of this study showed that VEGF immunohistochemistry could detect MI in decomposed specimens with no statistically difference from fresh myocardium until DD 1 and DD 2; until now, this is the first study which examined the potential of immunohistochemistry in decomposed forensic pathology specimens in tropical climate environment. It can demonstrate that immuno-histochemistry can be employed in decomposed specimens in tropical climate.

One limitation of this study is that only one piece of myocardium was prepared for microscopic examination for each DD due to the limited area of infarcted myocardium on gross specimens. In routine forensic pathology caseworks, multiple pieces of myocardium are prepared for microscopic examination. Hence, if multiple pieces of myocardium could be studied for each decomposition days, the result might be different.

Further researches on this topic should explore *in vivo* decomposed specimens as all the studies in this topic, including this study, had performed on specimens that undergone *in vitro* decomposition.[8, 9] As multiple immunohistochemistry markers should be used in diagnosis of MI, since each marker is suitable for different stages of myocardial infarction [12 - 24], more markers should be studied for decomposed MI specimens. So far, only C5b-9 (m) and NP57 have been studied [8, 9] and only this study has been performed on tropical climate environment. Furthermore, the possibilities of immunohistochemistry and other pathological lesions in Thailand and other tropical climate forensic caseworks should be explored.

Conclusion

This study is the first study which show that immunohistochemistry can be used in decomposed forensic specimens in tropical climate areas. Although VEGF could not be detected with significant difference from fresh specimen up to decomposition period of 2 days, in strongly positive cases, VEGF still persisted

at decomposition period up to 5 days. Further researches should explore more markers for myocardial infarction in decomposed cases together with the possibilities of immunohistochemistry and other decomposed forensic caseworks in tropical climate areas.

Acknowledgements

This research is granted by *Rachadapisaek-sompote Fund Grant No RA 60/056*.The authors would like to express our appreciation for all the staff members of the Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University for the specimen processes and preparations. Also, this work would not be achieved without the assistance of staff members of the Institute of Pathology, Department of Medical Services, Ministry of Public Health for their contribution on immunohistochemistry. This manuscript would not be grammatically correct for publication without the assistance of Mano Mettanando Laohavanich, MD, PhD.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Byard RW, Tsokos M. The challenges presented by decomposition. Forensic Sci Med Pathol 2013;9:135-7.
2. Byard RW, Farrell E, Simpson E. Diagnostic yield and characteristic features in a series of decomposed bodies subject to coronial autopsy. Forensic Sci Med Pathol 2008;4: 9-14.
3. Radheshi E, Reggiani BL, Confortini A, Silingardi E, Palmiere C. Postmortem diagnosis of anaphylaxis in presence of decompositional changes. J Forensic Leg Med 2016;38:97-100.
4. Kibayashi K, Hamada K, Honjyo K, Tsunenari S. Differentiation between bruises and putrefactive discolorations of the skin by immunological analysis of glycophorin A. Forensic SciInt 1993;61:111-7.
5. Tabata N, Morita M. Immunohistochemical demonstration of bleeding in decomposed bodies by using anti-glycophorin A monoclonal antibody. Forensic SciInt 1997; 87:1-8.
6. Omalu BI, Mancuso JA, Cho P, Wecht CH. Diagnosis of Alzheimer's disease in an exhumed decomposed brain after twenty months of burial in a deep grave. J Forensic Sci 2005; 50:1453-8.
7. MacKenzie JM. Examining the decomposed brain. Am J Forensic Med Pathol 2014;35: 265-70.
8. Thomsen H, Held H. Susceptibility of C5b-9(m) to postmortem changes. Int J Legal Med 1994; 106:291-3.
9. Ortmann C, Pfeiffer H, Brinkmann B. Demonstration of myocardial necrosis in the presence of advanced putrefaction. Int J Legal Med 2000; 114:50-5.
10. Zhou C, Byard RW. Factors and processes causing accelerated decomposition in human cadavers - An overview. J Forensic Leg Med 2011;18:6-9.
11. World Health Organization. The top 10 causes of death [Internet]. 2017 [cited 2017 Dec 20]. Available from: http://www.who.int/

mediacentre/factsheets/fs310/en/.

12. Campobasso CP, Dell'Erba AS, Addante A, Zotti F, Marzullo A, Colonna MF. Sudden cardiac death and myocardial ischemia indicators: a comparative study of four immunohistochemical markers. Am J Forensic Med Pathol 2008;29:154-61.
13. Sabatasso S, Mangin P, Fracasso T, Moretti M, Docquier M, Djonov V. Early markers for myocardial ischemia and sudden cardiac death. Int J Legal Med 2016;130:1265-80.
14. Mondello C, Cardia L, Ventura-Spagnolo E. Immunohistochemical detection of early myocardial infarction: a systematic review. Int J Legal Med 2017;131:411-21.
15. Ambade VN, Godbole HV, Batra AK. Atherosclerosis: a medicolegal tool in exhumed decomposed bodies. Am J Forensic Med Pathol 2008;29:279-80.
16. Xu XH, Chen JG, Zhu JZ. Primary study of vascular endothelial growth factor immunohistochemical staining in the diagnosis of early acute myocardial ischemia. Forensic SciInt 2001; 118:11-4.
17. Zhu BL, Tanaka S, Ishikawa T, Zhao D, Li DR, Michiue T, et al. Forensic pathological investigation of myocardial hypoxia-inducible factor-1 alpha, erythropoietin and vascular endothelial growth factor in cardiac death. Leg Med (Tokyo) 2008;10:11-9.
18. Crafts TD, Jensen AR, Blocher-Smith EC, Markel TA. Vascular endothelial growth factor: therapeutic possibilities and challenges for the treatment of ischemia. Cytokine 2015; 71:385-93.
19. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9: 669-76.
20. Cochain C, Channon KM, Silvestre JS. Angiogenesis in the infarcted myocardium. Antioxid Redox Signal 2013;18:1100-13.
21. Kobayashi K, Maeda K, Takefuji M, Kikuchi R, Morishita Y, Hirashima M, et al. Dynamics of angiogenesis in ischemic areas of the infarcted heart. Sci Rep 2017;7:7156.
22. Messadi E, Aloui Z, Belaidi E, Vincent MP, Couture-Lepetit E, Waeckel L, et al. Cardioprotective effect of VEGF and venom VEGF-like protein in acute myocardial ischemia in mice: effect on mitochondrial function. J Cardiovasc Pharmacol 2014;63: 274-81.
23. Siddiqui AJ, Fischer H, Widegren U, Grinnemo KH, Hao X, Mansson-Broberg A, et al. Depressed expression of angiogenic growth factors in the subacute phase of myocardial ischemia: a mechanism behind the remodeling plateau? Coron Artery Dis 2010; 21:65-71.
24. Zhao T, Zhao W, Chen Y, Ahokas RA, Sun Y. Vascular endothelial growth factor (VEGF)-A: role on cardiac angiogenesis following myocardial infarction. Microvasc Res 2010; 80:188-94.
25. Zhang W, Zhao X, Xiao Y, Chen J, Han P, Zhang J, et al. The association of depressed angiogenic factors with reduced capillary density in the Rhesus monkey model of myocardial ischemia. Metallomics 2016;8: 654-62.
26. He W, James KY. Ischemia-induced copper loss and suppression of angiogenesis in the pathogenesis of myocardial infarction. Cardiovasc Toxicol 2013;13:1-8.