

# Histochemical Characteristics of Myocardium Obtained from Two huge Cardiomegaly with over 1000g in Weight

Satoshi Furukawa<sup>1,2\*</sup>, Mayumi Kataoka<sup>1</sup>, Satomu Morita<sup>1,2</sup>, Akari Uno<sup>2</sup>, Masahito Hitosugi<sup>2</sup>, Hiroshi Matsumoto<sup>1,3</sup> and Katsuji Nishi<sup>1,2</sup>

<sup>1</sup>Osaka Medical Examiner Office, Osaka city, Japan

<sup>2</sup>Department of Legal Medicine, Shiga University of Medical Science, Japan

<sup>3</sup>Department of Legal Medicine, Osaka University, Japan

## Article Information

Received date: Feb 16, 2016

Accepted date: Feb 25, 2016

Published date: Feb 29, 2016

## \*Corresponding author

Satoshi Furukawa, The department of Legal Medicine, Shiga University of Medical Science, Japan,  
Email: 31041220@belle.shiga-med.ac.jp

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**Keywords** Huge Cardiomegaly;  
Hypoxia; Conventional Stain;  
Immunohistochemistry

## Abstract

We encountered two autopsy cases of huge cardiomegaly with over 1000g in weight. Histochemical characteristics were examined using conventional staining including HE and Azan stains and immunohistochemical staining using antibodies against Complement Component 9 (CC9), RNA Binding Protein Motif 3(RBM3), Endothelial Type NO Synthase (eNOS) and Hypoxic Inducible Factor1 $\alpha$  (HIF1). The reactive area with anti CC9 antibody, which presumed to be a marker of hypoxic change of myocardium, overlapped with eosinophilic area by HE and basophilic one by Azan. Although the reactive area with anti CC9 antibody showed relatively weak in the cytoplasm by anti eNOS antibody and no in the nucleus of cardiocytes with anti RBM3 antibody, outside of the reactive area with anti CC9 antibody there were intensive reactivity with anti eNOS antibody in cytoplasm and in a nucleus with anti RBM3 antibody. Anti HIF1 antibody showed weak reactivity with cytoplasm of endocardial cardiocytes and no with cytoplasm of cardiocytes in another area. The results obtained from the present cases revealed that the hypoxic change was equivalent even though the cause of cardiomegaly was deferred between two cases, and conventional staining such as HE and Azan utilized to detect hypoxic change in the heart and immunohistochemical studies seemed to be a useful tool for clarifying the cascade of hypoxic changes in the myocardium.

## Introduction

Cardiac hypertrophy can occur when factors, such as hypertension, aortic valve stenosis, amyloid deposition with ageing, and some genetic effects, makes one's heart work harder than normal to pump blood to one's body [1-3]. Cardiac hypertrophy is a compensatory mechanism of the heart, as it is critical for the maintenance of normal contractile function in response to chronic increases in hemodynamic load. Hypertrophy can initially be viewed as a salutary response; ultimately, however, it often heralds decompensation and transition to heart failure [4].

In a previous report, we described an autopsy case where a 92 year-old female died due to cardiomegaly caused by senile amyloid deposition in her heart [5]. The incidents of heart weights in human over 1000 g is exceedingly rare, since there were only 4 of 5845 victims, 4204 male and 1641 female adults, autopsied under normal condition without corruption change at Osaka Medical Examiner's Office during 1985 and 1994 [6]. In April, 2015 we encountered two individuals with huge cardiomegaly beyond 1000g at Osaka medical examiner's office and like to describe the histochemical characteristics of myocardium of them by means of conventional staining methods and immunohistochemical methods using antibodies against Complement Component 9 (CC9), RNA Binding Protein Motif 3(RBM3), Endothelial Type NO Synthase (eNOS) and Hypoxic Inducible Factor 1 $\alpha$  (HIF1).

## Case reports

### The first case

The Victim was a 63-year-old male with good physique and slight obesity. He worked as a furniture dealer and had received the treatment of heart failure due to aortic valve insufficiency. He took ACE inhibitor,  $\beta$ -blocker and diuretic drugs. He had no family history. One day, after eating dinner, he went his room; his corpse was found in his room. Intensive livor mortis was expressed on his back and also on his anterior neck area. The autopsy was performed on the next day. He was taller than usual and moderately heavy build with 2cm thickness of adipose tissue at abdominal wall. No edema was seen in his conjunctiva, face and lower extremities. All organ was severe congestive and slightly massive than usual. Six hazelnut-sized gallstones were detected in the gallbladder. The heart with intensive rigidity had about the size of a grab with hands by one of the authors and was a weight 1150g (Figure 1), and was typically the size of a small rugby ball. Its orthogonal dimensions were 16.5×11.5×11.5cm.



**Figure 1:** Figure 1: Appearance of the heart from 63 year-old male. The heart with intensive rigidity had about the size of a grab with hands by one of the authors (M K) and was a weight 1150g, and was typically the size of a small rugby ball. Its orthogonal dimensions were 16.5x11.5x11.5cm.

There was moderate macroscopic fibrosis on cross-section of the heart as shown in Figure 2, and the thickness of both side ventricles were remarkable, 3.3cm in left, 2.0cm in right and 3.4cm in septum. Severe calcification of aortic valves was recognized and it caused constriction of aortic arch to be 7.2cm at valves level. The weight of lung was measured 500g left and 650g right showing congestion lung, respectively.

**The second case**

The victim was 78 year-old male, had received the treatment due to hyperuricemia and hypertension. He took calcium antagonist, Angiotensin II Receptor Blocker (ARB), and gout suppressant. He had no family history. One day before finding his corpse he complained of chest pain and his corpse was found in his room on the next day. The autopsy was performed on the next day. He was taller than usual and moderately obesity with 4cm thickness of adipose tissue at abdominal wall. Moderate edema was seen in his conjunctiva, face and lower extremities.



**Figure 2:** Macroscopic finding of cross-section. There was moderate macroscopic fibrosis on cross-section of the heart as shown in Photo 2, and the thickness of both side ventricles were remarkable, 3.3cm in left, 2.0cm in right and 3.4cm in septum.



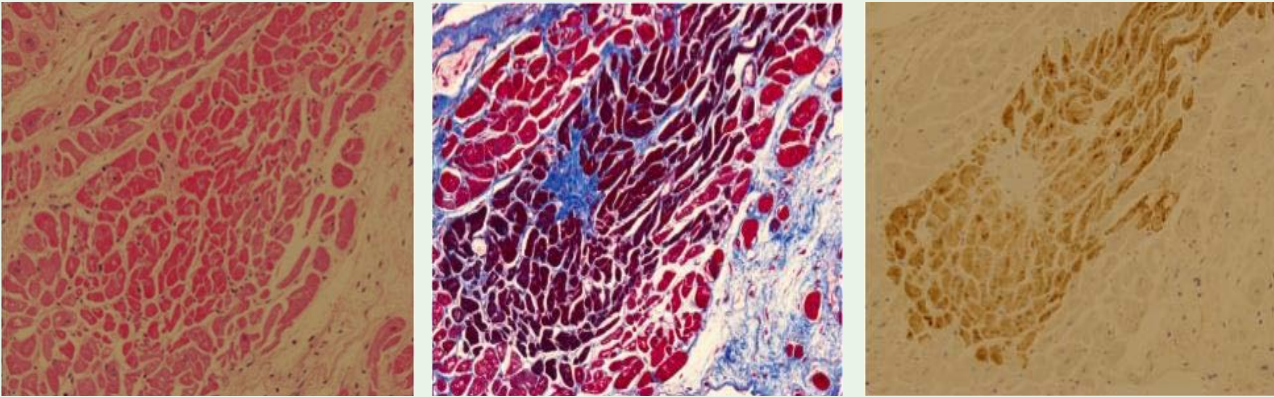
**Figure 3:** Appearance of the heart from 78 year-old male. The heart was flabby and enlarged due to postmortem change; it is typically the size of a giant hamburger bun: 19cm in length, 15cm wide, and 6.7cm in thickness and 1080g in weight.

All organ was severe congestive and more massive than usual, lung was weighted 690g in right and 800g in left. Although the heart was flabby and enlarged due to postmortem change (Figure 3), it is typically the size of a giant hamburger bun: 19cm in length, 15cm wide, 6.7 cm in thickness and 1080g weight. There was no remarkable and macroscopic fibrosis in the endocardium as shown in Figure 3, and the thickness of both side ventricles were remarkable, 1.9cm in left, 0.8cm in right and 2.8 cm in septum. The moderate degree arteriosclerosis was observed about 1cm to 2cm parts from left coronary artery proximal portion. However remarkable bleeding changes were not macroscopically detected. Severe aortic valve calcification was recognized on three valves (Figure 4) and the width of aortic arch was to be 7.5cm at valve level showing constriction.

The cause of cardiomegaly was deference between two cases. It was thought to be based on aortic insufficiency in the first case and on hypertension in the second case.



**Figure 4:** Macroscopic finding of left ventricular inside. There was no remarkable and macroscopic fibrosis in the endocardium and the thickness of both side ventricles were remarkable, 1.9cm in left, 0.8cm in right and 2.8 cm in septum. The moderate degree arteriosclerosis was observed about 1cm to 2cm parts from left coronary artery proximal portion. Sevier aortic valve calcification was recognized on three valves and the width of aortic arch was to be 7.5cm at valve level showing constriction.



**Figure 5:** Staining findings by HE, Azan and anti CC9 antibody. The staining findings were similar and indicated that areas of eosinophilic by HE, blue purple by Azan and Brown by anti CC9 antibody result from hypoxic change. Fibrotic area in Azan stain was not stained by anti CC9 antibody.

**Microscopic finding**

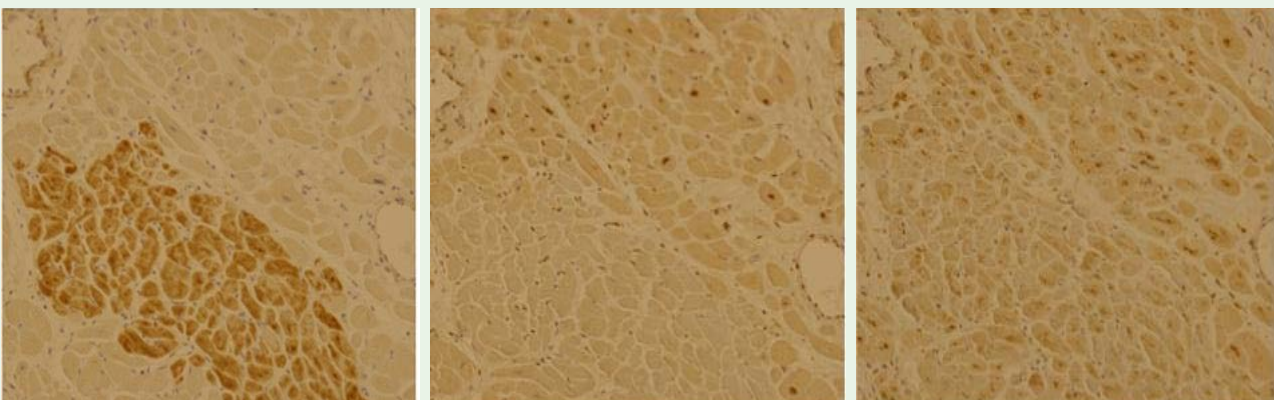
Tissue blockages of the both heart were sectioned and embedded in paraffin. Tissue sections of 3µ thickness were stained by HE, Azan and congo red methods and reacted with antibodies against HIF1α, eNOS, RBM3 and CC9, respectively. The staining procedure and antibodies were identical to our previous report [7]. After staining the sections were observed and made photos by the Nikon microscopic system. Microscopic findings obtained from both case showed almost the same and the results were described together.

(1) Eosinophilic area by HE and basophilic area by Azan overlapped reactive area by anti CC9, eNOS and RBM3 antibodies. At microscopic level, Azan stain revealed large (case 1) and small (case 2) amount of fibrotic area, reddish or dark blue changed areas mainly in the left ventricle. The intensive reactive area with anti CC9 antibody was observed in the left ventricle tissue and this area exactly overlap each other with dark blue changed area (basophilic area) by Azan stain and with eosinophilic area by HE. In the surrounding area of the reactivity by anti CC9 antibody, many myocardial cells showed the good reactivity by anti RBM3 antibody in the nucleuses. The anti eNOS antibody stained moderately inside and intensively outside of

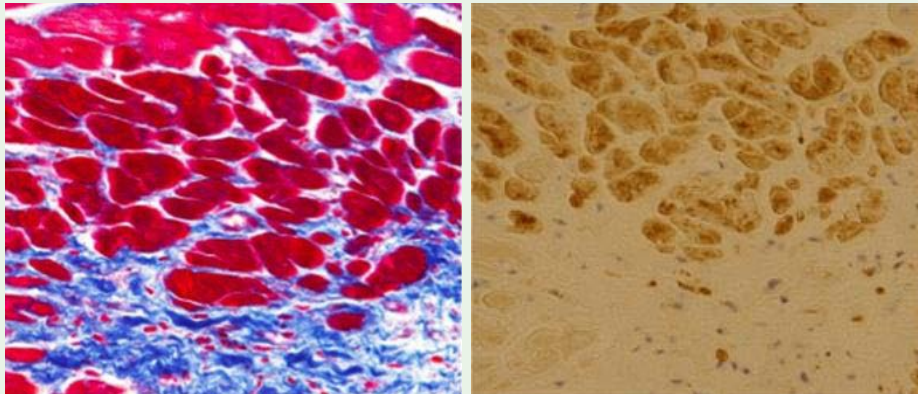
the anti CC9 antibody positive area, making some granules in the cytoplasm. In the chronic fibrotic area, blue changed area by Azan stain, no reactivity with antibodies against RBM3, eNOS and CC9, although the reactivity with anti eNOS and RB3 antibodies on the macrophages. Outside of the chronic fibrotic area where normal cells existed, remarkable reactivity with anti RBM3 and eNOS antibodies were recognized in the nucleuses and cytoplasm, respectively. No reactivity by anti CC9 antibody was not detected (Figure 5).

(2) Eosinophilic area by Azan stain and reactivity of the area by anti CC9 antibody. In the reddish changed area by Azan stain there were several myocardial cells containing contraction bands shown by wavy lines with dark orange. In this area the reactivity with anti CC9 antibody was irregular, that is, clear in some part and feeble in other part (Figure 6). In this area, anti eNOS antibody showed relatively good reactivity, but anti RBM3 antibody had lost the activity with the nucleus.

(3) Other findings Overall, microscopic abnormalities including myocyte hypertrophy, fibrosis, moderate disarray, and vacuolization were recognized with different degree in both cases; however there was no amyloid deposition in the myocardium from both cases.



**Figure 6:** Staining findings by antibodies against CC9, RBM3 and eNOS. Anti CC9 antibody stained hypoxic area and arterial blood vessels but not venous vessels. Anti RBM3 antibody stained nucleus in the cardiocytes existed in surroundings of hypoxic area and blood vessel of artery and vein. Although anti eNOS antibody stained weakly cytoplasm of cardiocytes in the hypoxic area, intensive reactivity was observed with those of existing in surrounding of hypoxic area, and blood vessel.



**Figure 7:** Contraction bands like figure in the hypoxic area by Azan stain. A large amount of clear red lines was recognized in the cardiocytes existed in hypoxic area. On right slide, porphyritic staining by anti CC9 antibody was observed in the cardiocytes.

Anti eNOS and RBM3 antibodies showed good reactivity with endothelial cells of arteries and veins, however anti CC9 antibody showed reactivity only with those of arteries (Figure 7). The reactivity with anti HIF1 $\alpha$  antibody were detected in the cytoplasm of some endocardial cells but not inside and outside of the area stained by anti CC9 antibody, and no relation of the reactivity among anti HIF1 $\alpha$ , eNOS and RBM3 antibodies.

## Discussion

It is well acceptance that eosinophilic change of myocardium by HE stain was occurred due to ischemic change of the heart tissue; however there was no confirmation until now. Although it has been assumed that liver is the primary source of complement components, Yosojima, et al. [8] reported that the mRNAs and proteins for complement C3 and C9 were also expressed in rabbit heart. They revealed that hearts subjected to 0.5 hours of ischemia followed by 1 hour of reperfusion had a 19.5-fold increased in C9 mRNA and described that cardiomyocytes and endothelial cells were obvious candidates. Ortman, et al. [9] described that anti CC9 antibody was most useful to detect the early hypoxic changes in the heart. The reactivity of anti CC9 antibody indicates early hypoxic change in the present cases. The overlapped color changes by HE, Azan and anti CC9 antibody staining may be due to early damaged area.

Anti CC9 antibody showed irregular reactivity in eosinophilic area by Azan stain. We previously reported that orange color changing like present results in the myocardium was detected in the heart tissue obtained from hypothermic death individuals, and anti eNOS and RBM3 antibodies stained the cell cytoplasm or nucleuses existed the color changed and surrounding area, showing these cells able to keep their ability to promote the cascade.

The expression of these antigens might be stipulated by the agonal duration of individual [7]. It is well known that the NO together with cGMP activates the ATP specific K-channel of the sarcolemma to attenuate the hypertrophic response in hyperlipidemic models [10]. It is also well accepted that NO induces the activation of caspases, DNA fragmentation and apoptosis in high concentration whereas in low concentration NO inhibits the cardiomyocyte hypertrophy [11]. Weak reactivity with anti-eNOS antibody in the basophilic area by Azan stain might due to be going to apoptotic stage, and no reactivity

with anti-RBM3 might due to damaging myocardial cells that have already lost their protective cascade pathway. On the other hand outside of the basophilic area by Azan, eNOS and RBM3 might be effectively expressed to protect the ischemic death of cardiomyocytes.

The results together with previous one show that the myocardial cells with orange color by Azan stain may be in very early ischemic stage. In this examination reactivity of anti CC9 antibody were detected in endothelial cell of blood vessels and selected area in the myocardium, where the myocardium showed different color compared with surrounding area by conventional staining and weak reactivity with anti eNOS and RBM3 antibodies.

Although reactivity of blood vessels by anti eNOS and RBM3 antibodies was almost similar, anti CC9 antibody was able to stain the arterial blood vessels. It has been recognized in our previous research [12] that the RBM3 and eNOS were expressed in endothelial cells from the both of arteries and veins. At present there is no information and idea to solve these different findings.

The reactivity with anti HIF1 $\alpha$  were detected in the cytoplasm of some endocardial cells but not inside and outside of the area stained by anti CC9, and no relation of the reactivity among anti HIF1 $\alpha$ , eNOS and RBM3 antibodies. The present study confirms the reports from Wellmann, et al. [13]. The expression of HIF1 $\alpha$  seems to be delayed or slowly than those of other hypoxic antigens. HIF1 $\alpha$  is usually expressed in cytoplasm, and constitutively decomposed by protease, however it appears in cytoplasm and/or nucleus when continued hypoxia and reperfusion [14].

## Acknowledgement

The author's work was partially supported by a Grant-in-Aid Scientific Research C (2640876) for K Nishi.

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