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Assessment of myocardial vasculature in chronic alcoholics without established cardiomyopathy

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Abstract Myocardial pathology and cardiac functional abnormalities known to occur in association with chronic alcohol abuse have been attributed to a direct toxic effect of ethanol on the intra-myocardial vasculature: alcohol-induced occlusion of small arteries with consequent secondary ischaemia leads to individual myocyte loss, focal fibrosis and compensatory cardiac hypertrophy. To assess the intra-myocardial arterial vessels an interactive semi-automated, computerised, high-resolution, video image-analysing system was used and the findings from hearts of alcoholics were compared with normal hearts collected at autopsy. The elastic laminae, stained with Van Gieson's stain, acted as the reference points for measurement of the diameters, circumferences and thicknesses of the vessels assessed. It was not possible to demonstrate any statistically significant morphometric changes within intra-myocardial vasculature. The same technique used is readily adaptable for the assessment of the blood vessels in other organs and tissues.

Key words Alcoholism · Myocardium · Blood vessels · Morphometry · Image analysis

Introduction

Most pathology studies of alcoholic heart disease have focused on the light and electron microscopic alterations of the cardiac muscle fibres [1–8]. One of the hypotheses which have been put forward to explain the pathogenesis of these lesions is chronic ischaemia and as degenerative disease of the main coronary arteries is not usually a feature found in chronic alcoholics – rather vice versa – the proposal advanced was that these changes are mediated by disease of the smaller coronary vessels within the myocardial interstitium itself [9].

This morphometric study of the intra-myocardial vasculature in chronic alcoholics was carried out to evaluate any vascular changes that could be attributed to chronic alcoholism. So as to assess this condition in the absence of any other confounding abnormalities, it was decided to choose hearts from patients with well-documented severe chronic alcohol abuse but which showed no gross morphological features attributable to this habit. In this way it was also anticipated that only the early changes of any vascular abnormalities would be identified.

Materials and methods

The hearts which were selected for this study could be subdivided into two groups – "the study group" from decedents known to be chronic alcohol abusers, and "the control group" from non-alcohol abusers. All the hearts were collected randomly for this study from persons dying suddenly and unexpectedly who had been referred for forensic autopsy during the years 1991–1993. The criteria which the "control group" had to fulfill were the following: no evidence in the medical history that the deceased was a chronic heavy smoker, or suffered from systemic hypertension, respiratory (e.g. chronic obstructive airway disease) or cardiac problems, or had a history of chronic alcohol abuse. The examination of the deceased's domicile at the time of death of the deceased by the police, social workers' reports (when available) and evidence from relatives or friends whom the police interviewed were used to exclude heavy chronic consumption of alcohol. At autopsy there was no evidence of heavy smoking and the heart on inspection showed no gross structural or valvular abnormalities. Careful sectioning of the coronary arteries showed no areas of stenosis in excess of 30% of the lumen. At autopsy and subsequent extensive histological examination of the internal organs, no pathology was found which could have had a bearing on the heart.

In the selection of the hearts for the "study group", the same general criteria had to be satisfied and that there had to be good evidence of chronic alcohol abuse with the diagnosis being based on the police "sudden death report" (sent under confidential cover to the local investigating legal official, the Procurator Fiscal), on the medical history which was appended to the police report and had

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been obtained by interviewing the family medical practitioner, the family of the deceased and any other persons who knew the deceased during life. Cases in which there was macroscopically evident cardiomyopathy, with such features as a combination of marked enlargement of the heart associated with marked multichamber dilatation with or without mural thrombus formation or a pale and flabby myocardium with a variegated appearance, were excluded. Any of the hearts that on section showed any ischaemic damage were also discarded. Most of these deceased showed varying degrees of fatty infiltration of the liver but cirrhotic patients were excluded.

A total of 68 hearts derived from autopsies were studied: 40 from chronic alcoholics and 28 from adult non-alcoholics. In each case a full autopsy was carried out by a consultant forensic pathologist at which all the body cavities were examined, toxicological studies carried out where indicated (including an assay of alcohol in the blood, urine and vitreous fluid) and specimens from most organs subjected to histological examination. Once the heart was removed, the coronary arteries were examined carefully by transverse sequential cuts at 2–5 mm intervals. The main vessels of the heart were trimmed away close to the transverse pericardial sinus and the heart was opened in a serial transverse circumferential method at 1 cm intervals. After fixation in 10% buffered formalin, three macroscopical slices were chosen from the heart specimens i.e. from the proximal, middle and distal third of the heart at approximately 2 cm, 4 cm and 6 cm respectively from the atrio-ventricular ring. Histological blocks were taken from the anterior wall of the left ventricle (from proximal third), the ventricular septum, the anterior wall of the right ventricle and the lateral wall of the left ventricle (from middle third) and the posterior wall of the left ventricle (from distal third). After post-fixing in 10% buffered formalin the blocks were embedded in paraffin wax, and 3–4 μm thick sections were cut in a standard fashion. The slides were stained with haematoxylin and eosin (H&E) and Van Gieson's elastica stain.

To measure different variables of blood vessels, a program was designed and a computerised inter-active image analysis system (IBAS) was used. This multi-purpose image-analysis workstation consisted of a Leitz Ortholux-2 microscope, coupled to a high resolution black & white Sony CCD xc 77CE video camera. The video image was inputted to a Kontron AT (KAT) image analysis system (IBM compatible) computer, coupled with a Kontron visual display unit (program monitor) plus a MIAP2 image processing unit linked with a high resolution RGB (i.e. red, green, blue) monitor, a digital tablet and stylus, a keyboard, and a list printer. A video printer could also be connected to take the photographs. The results were shown to be reproducible with the margin of error being limited and within acceptable scientific limits as shown in pilot studies.

To measure blood vessels, slides which had been stained by Van Gieson's elastica stain were used, because the elastic tissue in this stain stained as a black or purplish black wavy line. The specific measurements which were taken were limited to arterial vessels and only to those which were sectioned in a roughly transverse direction; vessels which exhibited tangential or longitudinal cuts were excluded from the study with the vessels measured being therefore largely of a rounded or oval configuration. For those vessels which were not sectioned in the apparently transverse plane, a mathematical adjustment was made for this artefact.

A correction formula was introduced which enabled measurements obtained on vessels which appeared elongated or oval on section to be converted into measurements for similar hypothetical circular vessels. The diameter of a hypothetical circle with circumference equivalent to an ellipse was calculated using the formula: $(\text{equivalent diameter})^2 = [(\text{min. diameter})^2 + (\text{max. diameter})^2]/2$. Another correction formula was used to measure the degree of collapse and to examine the shape of the blood vessels by calculating the eccentricity of the hypothetical ellipse by expressing the minimum diameter as a percentage of the maximum diameter. The formula used was: eccentricity = (minimum diameter/maximum diameter) × 100. This gives 100% for circular vessels and smaller values for very elongated shape.

The slides were viewed under the × 25 objective lens and the images were digitised into 512 × 512 pixels on the IBAS machine using the grey scale ranging from 0 to 255 (black to white). Analysis and processing of the digitised images were carried out by a sequence of macro-routines specifically tailored to assess the variables.

The vessels were selected randomly by scanning the slide and ten vessels per slide which had intact internal elastic lamina (IEL) were measured. The internal diameter of a blood vessel was considered to be represented by the IEL for this study and the IEL was also considered as a standard variable in this study to determine the size of the vessel. If the outline of the external elastic lamina (EEL) could not be seen, and at least 7/8ths of intact EEL were not clearly visible then that particular vessel was not measured. In these randomly selected blood vessels the following vascular measurements were carried out: the total circumferential length of the IEL, the area of the vessel encircled by IEL, the maximum and minimum diameters of IEL enclosed space (measured automatically by careful manipulation of the cursor and adjustment of the colour scale). It was possible to selectively identify the IEL as such as it always produced a higher level of colour intensity and could be specifically selected out. In taking such measurements the IEL was encircled with the light pen to exclude positively by direct interaction the areas occupied by tissues which, although showing up as having similar colour intensity threshold values as IEL, were not to be measured, and these unwanted objects and other components of vessel wall were rejected.

The total area occupied by the lumen of blood vessels and its maximum and minimum diameters were measured automatically by the machine by manipulating the cursor to choose the appropriate grey levels on the grey scale using threshold values; the grey levels selected were overlaid with a green overlay. The area occupied by the intima, and maximum and minimum width of the intima of the blood vessels were calculated automatically by the machine by subtracting the luminal area from the area covered by the IEL, and the luminal diameters from the diameters of the IEL as programmed. In the smaller vessels it was not possible to distinguish endothelial cells from the IEL and therefore intimal measurements in these vessels included IEL.

The circumferential length, maximum and minimum diameters and the area of the vessel wall encircled by EEL were measured by carefully tracing the EEL with the light pen, and this allowed automatic calculation of these variables by the machine. The total area of the media and the maximum and minimum width of the media were calculated automatically by the machine by subtracting the area encircled by the IEL from the area encircled by the EEL and the diameters of the vessel at the level of the IEL from the diameters obtained at the level of the EEL.

Statistical comparison of the two groups was carried out by analysis of covariance.

Results

The total circumferential length of IEL was measured and a comparison made between measurements of circumferential length of IEL in the different regions of the hearts from the alcoholics and controls as shown in Table 1. Apart from the measurements in the lateral wall of the left ventricle, the vessels assessed in other regions of the hearts were of comparable size. Subsequent comparisons were adjusted for mean length of IEL in order to ensure that vessels of similar size in both alcoholics and controls were compared as the length of the IEL was taken as a standard variable in this study to measure the size of the blood vessels. This adjustment also gives an increase in statistical power since the length of the IEL is correlated with most of the measurements carried out in this study. Comparisons of the diameters of the area bounded by the IEL were made between the two groups. After adjustment

Table 1 Mean (sd) circumferential length of the internal elastic lamina measurement (μm). (Significance refers to result of two-sample t-test for group difference)

Region	Alcs.	Ctrls.	Sig.
Ant. wall of rt. vent.	99.70 (17.58)	103.27 (21.99)	NS
Ant. wall of lt. vent.	108.83 (26.08)	100.38 (26.37)	NS
Lat. wall of lt. vent.	109.32 (26.33)	94.59 (17.01)	$P < 0.01$
Ventricular septum	126.22 (35.91)	113.30 (29.74)	NS
Post. wall of lt vent.	112.70 (26.75)	108.19 (23.09)	NS

sd – standard deviation; alcs. – alcoholics; ctrls. – controls; sig. – significance; ant. – anterior; rt. – right; vent. – ventricle; lt. – left; post. – posterior; NS – not significant; IEL – internal elastic lamina; EEL – external elastic lamina

for the length of the IEL no statistically significant difference was identified in relation to the maximum and minimum diameters of the IEL between the two groups (Table 2).

To assess any difference in the size of lumen of blood vessels from alcoholics as compared to controls, the total area of the lumen and its maximum and minimum diameters were measured (μm). Alcoholics showed no statistically significant difference in the measurement of area of the lumen of blood vessel as compared to controls. The only statistically significant difference was identified in the right ventricle and the septum of the left ventricle, with all other areas being non-significant. The results are listed in Tables 3 and 4.

The mean values for the total area of intima and it's thickness (although the short-hand term "diameter" is used throughout) are shown in the Tables 3 and 4. These comparisons show that there is no statistically significant difference between 2 groups as far as the area of intima is concerned, thus indicating that no intimal thickening of blood vessels was found in either the left ventricle or the right ventricle in alcoholics as compared to non-alcoholic controls. As already discussed, the external diameter of a blood vessel is considered to be represented by the EEL in this study. The total circumferential length of the EEL and it's maximum and minimum diameters were compared between the two groups and only the results for the diameters are listed in Table 2, as the EEL circumferential length is known not to be as constant variable as the IEL. Alcoholics showed no significant difference in their maximum and minimum diameters of the EEL as compared with controls. In the comparisons of the measurement of total area and diameters of the media between the two groups the only statistically significant difference was shown in the anterior and lateral wall of the left ventricle where alcoholics showed increased medial area (Table 3) as compared with controls. As far as the diameter of media is concerned, there was no significant difference for the calculated figures obtained for the alcoholics as compared to controls (Table 4).

Overall 65 significance tests were carried out, of which 5 were significant at the 5% level and one at the 1% level: this is approximately what would be expected by chance alone, and suggests that there are no consistent differences between alcoholics and controls in the dimensions of their blood vessels after standardisation for length of IEL.

Table 2 Mean (sd) maximal and minimal diameters of the IEL and EEL measurement (μm). (Significance refers to two-sample t-test for group difference adjusted for mean length of IEL)

Region	Maximum diameter			Minimum diameter		
	Alcs.	Ctrls.	Sig.	Alcs.	Ctrls.	Sig.
IEL measurement						
Ant. wall of rt. vent.	33.87 (5.92)	34.65 (7.28)	NS	23.48 (4.18)	23.90 (4.51)	NS
Ant. wall of lt. vent.	37.85 (9.56)	34.50 (8.00)	NS	23.82 (4.51)	22.32 (6.22)	NS
Lat. wall of lt. vent.	37.96 (9.38)	32.43 (5.84)	NS	24.14 (4.55)	21.67 (3.83)	NS
Ventricular septum	42.71 (11.98)	38.54 (9.36)	NS	27.67 (6.86)	25.77 (6.15)	NS
Post. wall of lt. vent.	37.05 (7.45)	36.96 (7.90)	NS	26.29 (6.65)	24.48 (4.57)	NS
EEL measurement						
Ant. wall of rt. vent.	45.16 (7.75)	45.83 (9.89)	NS	32.54 (5.75)	33.30 (7.14)	NS
Ant. wall of lt. vent	48.40 (10.97)	45.29 (10.35)	NS	32.53 (5.90)	30.63 (8.01)	NS
Lat. wall of lt. vent	48.69 (11.03)	42.39 (6.84)	NS	32.73 (6.00)	29.68 (5.27)	NS
Ventricular septum	54.30 (14.02)	49.92 (12.92)	NS	38.49 (9.32)	35.46 (9.28)	NS
Post. wall of lt. vent	48.69 (9.50)	47.48 (9.47)	NS	34.99 (7.39)	33.31 (6.02)	NS

sd – standard deviation; alcs. – alcoholics; ctrls. – controls; sig. – significance; ant. – anterior; rt. – right; vent. – ventricle; lt. – left; post. – posterior; NS – not significant; IEL – internal elastic lamina; EEL – external elastic lamina

Table 3 Mean (sd) luminal, intimal and medial area measurement (μm^2). (Significance refers to two-sample t-test for group difference adjusted for mean length of IEL)

Region	Alcs.	Ctrls.	Sig.
Luminal area			
Ant. wall of rt. vent.	468.94 (213.55)	512.03 (327.73)	NS
Ant. wall of lt. vent	531.41 (338.47)	478.23 (469.34)	NS
Lat. wall of lt. vent	525.56 (302.15)	407.89 (289.65)	NS
Ventricular septum	744.69 (566.24)	610.41 (393.91)	NS
Post. wall of lt. vent	625.95 (433.66)	532.15 (266.85)	NS
Intimal area			
Ant. wall of rt. vent.	219.68 (62.04)	239.82 (71.27)	NS
Ant. wall of lt. vent	262.20 (95.70)	226.73 (72.95)	NS
Lat. wall of lt. vent	260.49 (127.72)	215.43 (63.25)	NS
Ventricular septum	336.82 (146.73)	287.73 (128.14)	NS
Post. wall of lt. vent	273.00 (97.71)	260.80 (83.10)	NS
Medial area			
Ant. wall of rt. vent.	638.59 (280.43)	713.01 (474.81)	NS
Ant. wall of lt. vent	643.57 (380.66)	614.87 (442.38)	$P < 0.05$
Lat. wall of lt. vent	639.01 (333.01)	558.38 (296.23)	$P < 0.05$
Ventricular septum	902.66 (487.42)	831.83 (608.10)	NS
Post. wall of lt. vent	701.17 (295.71)	661.29 (337.15)	NS

sd – standard deviation; alcs. – alcoholics; ctrls. – controls; sig. – significance; ant. – anterior; rt. – right; vent. – ventricle; lt. – left; post. – posterior; NS – not significant; IEL – internal elastic lamina; EEL – external elastic lamina

Discussion

Several clinical and experimental studies have pointed to the existence of small vessel disease in chronic alcoholism. In a study of chronic alcoholics, oedema of the wall of coronary vessels was described in an early stage of alcohol-induced cardiac disease [10]. A marked increase in vascular permeability together with changes in vascular tone has also been found: this increased vascular permeability resulted in interstitial oedema of both the myocardium and the vessel walls, and it was suggested that the protein-rich plasma which accumulated between the layers of the arterial walls produced gradual narrowing and distortion of the vessel lumen which progressed to complete occlusion, eventually resulting in subsequent focal myocardial ischaemic necrosis and focal minimal scarring [11]. Somewhat similar changes have been produced experimentally in dogs rendered hypomagnesemic over a long period of time [12]; hypomagnesemia has been reported in chronic alcoholics and this has been advocated as one of the potential mechanisms for myocardial toxicity [13]. Pintar et al. [14] demonstrated significant changes in small intra-myocardial vessels of three chronic alcoholics dying of cardiomyopathy: these included vascular oedema and degeneration, disorganisation of the vessel wall layers and deposition of PAS-positive material in the subintima [14]. In the study of Sohal et al. mice fed on a diet containing 15% ethanol by volume for 3 months showed ultrastructural changes in myocardial capillaries consisting of swollen and degenerating endothelial cells and narrowing of the vascular lumen: the role of chronic hypoxia induced by these vascular alterations was emphasised as playing a role in the pathogenesis of alcoholic heart disease [15]. The small coronary arteries of two patients with alcoholic cardiomyopathy were studied by fine particle barium injection and soft X-ray technique: the spatial architecture of the small arteries, which penetrated the depths of the right and left ventricular myocardium, were found to be normal: their luminal surfaces were smooth and no major abnormalities apart from some luminal dilatation were found [16].

In the study of the hearts of nine chronic alcoholics under 45 years old, vascular changes were tabulated into the various categories of vascular oedema, sclerosis, peri-vascular fibrosis, inflammation and sub-endothelial 'humps' to determine the significance and degree of microvascular alterations; other variables were noted that were not directly related to the vessels and these included the presence of interstitial fibrosis, areas of myocardial fibrosis, interstitial inflammation, epicarditis, recent or acute thrombosis of vessels and areas of acute myocardial infarction; a combination of these vascular changes were identified. In all these patients microscopic changes were identified [17]. Changes were also demonstrated in the small intra-myocardial vessels, particularly 'subendothelial humps' in a 72-year-old chronic alcoholic man who died suddenly with a heart weight of 325 g and showed no coronary arterial atheroma of significance [18].

Researchers have quantitated the structural components of blood vessels in different organs in several diseased states indirectly by mathematical calculation from micrometer measurements [19, 20] or directly by planimetry [21, 22]. However, there has always been a problem of finding a reliable indicator of vessel size to which the measurements could be related. The total circumferential length of IEL has been considered as a standard variable in this study in relation to other variables and the internal diameter of a blood vessel is considered to be represented by the IEL in this study, as the IEL has been shown to be the least affected variable in post-mortem fixation and slide preparation, and its total length remains unaltered even when the vessel collapses or constricts [23]. The use of this measurement was carefully validated in the study of medial hypertrophy in pulmonary arteries [24]. Other investigators have also defined artery size in terms of the length of the internal elastic lamina [25–27].

Table 4 Mean (sd) luminal, intimal and medial diameters measurement (μm). (Significance refers to two-sample t-test for group difference adjusted for mean length of IEL)

Region	Maximum diameter			Minimum diameter		
	Alcs.	Ctrls.	Sig.	Alcs.	Ctrls.	Sig.
Luminal diameters						
Ant. wall of rt. vent.	28.74 (5.67)	29.07 (7.06)	$P < 0.05$	18.71 (4.03)	18.84 (4.42)	NS
Ant. wall of lt. vent.	32.17 (9.31)	29.03 (8.01)	NS	18.65 (4.51)	17.43 (6.20)	NS
Lat. wall of lt. vent.	32.71 (9.05)	27.89 (6.51)	NS	19.12 (4.25)	16.26 (4.80)	NS
Ventricular septum	37.80 (12.62)	32.45 (8.88)	NS	21.99 (6.36)	20.50 (5.59)	$P < 0.05$
Post. wall of lt. vent.	32.23 (8.39)	31.27 (7.61)	NS	21.11 (6.50)	19.25 (4.46)	NS
Intimal diameters						
Ant. wall of rt. vent.	2.57 (0.25)	2.79 (0.31)	$P < 0.01$	2.40 (0.21)	2.53 (0.21)	$P < 0.05$
Ant. wall of lt. vent	2.78 (0.35)	2.74 (0.41)	NS	2.58 (0.41)	2.45 (0.19)	NS
Lat. wall of lt. vent	2.63 (0.31)	2.67 (0.34)	NS	2.51 (0.34)	2.45 (0.30)	NS
Ventricular septum	2.87 (0.40)	2.86 (0.46)	NS	2.84 (0.44)	2.63 (0.40)	NS
Post. wall of lt. vent	2.72 (0.39)	2.85 (0.46)	NS	2.59 (0.47)	2.62 (0.30)	NS
Medial diameters						
Ant. wall of rt. vent.	5.62 (1.20)	5.59 (1.71)	NS	4.54 (1.03)	4.68 (1.66)	NS
Ant. wall of lt. vent	5.33 (1.17)	5.39 (1.61)	NS	4.36 (1.17)	4.16 (1.07)	NS
Lat. wall of lt. vent	5.36 (1.23)	4.98 (0.97)	NS	4.30 (0.96)	4.00 (1.04)	NS
Ventricular septum	6.00 (1.37)	5.85 (2.17)	NS	5.16 (1.21)	4.85 (1.76)	NS
Post. wall of lt. vent	5.48 (1.17)	5.26 (1.36)	NS	4.35 (0.85)	4.41 (1.22)	NS

sd – standard deviation; alcs. – alcoholics; ctrls. – controls; sig. – significance; ant. – anterior; rt. – right; vent. – ventricle; lt. – left; post. – posterior; NS – not significant; IEL – internal elastic lamina; EEL – external elastic lamina

The study presented here is a detailed morphometric assessment of the intramyocardial vasculature in autopsy hearts: the study is largely free of observer bias, is comprehensive and reproducible. On the basis of measurements and calculations made of different variables of the arterial vascular channels e.g. area and diameters of lumen, intima and media etc., in the hearts of alcoholics, overall 65 tests of significance were carried out but there were only 5 significant results at the 5% level, and only one at the 1% level. This suggests that there are no consistent differences in thickening of the vascular walls of small intra-myocardial arterial vessels at least in hearts of alcoholics prior to the development of gross morphological changes of a cardiomyopathy. The only suggestion that can be made from the statistical analyses of the vessel wall measurements is that intra-myocardial arterioles show some tendency to dilatation and medial thickening was identified in two areas but little else of significance may be deduced from the other measurements. There appears to be no consistent abnormality in the intra-myocardial arteries and arterioles in the chronic alcoholics examined.

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