

Early Atherosclerotic Lesions in Sudden Infant Death Syndrome

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ABSTRACT

Objective

The aim of the present study was to assess the morphology of early atherosclerotic lesions in coronary arteries from sudden infant death syndrome (SIDS) victims in order to recognize atherogenic mechanisms.

Material and Methods

We examined 52 victims of SIDS and 16 controls with known causes of infant death. The principal coronary arteries were serially cut and stained with hematoxylin-eosin, Azan, Alcian blue, acetic orcein, CD68, CD34 and α -SM actin, with subsequent histomorphometric analysis of the lesions.

Results

Preatherosclerotic lesions were found in 44.2% in SIDS group (23/52) and only in 6.3% in controls (1/16) ($p=0.0062$). Smooth muscle cells lost polarity, infiltrating the subendothelium, with rupture of the internal elastic membrane (IEM) in most cases. Angiogenesis was not observed. When muscular and elastic intimal thickening was present in the SIDS group, the results were as follows: neointimal thickness, 58.3 ± 17.8 μ m; affected perimeter of the IEM, 45.6%; area of neointimal proliferation, 0.03 ± 0.01 mm²; and luminal area, 0.21 ± 0.1 mm² with a luminal stenosis of $13.8 \pm 5\%$.

Conclusions

Preatherosclerotic lesions develop early in SIDS victims, and they are significantly more frequent than in controls. Smooth muscle cells are fundamental in its genesis.

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Key words >

Atherosclerosis - Sudden Infant Death - Coronary Vessels

Abbreviations >

SMCs Smooth muscle cells

IEM Internal elastic membrane

PCNA Proliferating cell nuclear antigen

SDIS Sudden death infant syndrome

BACKGROUND

In 1955, Enos, Holmes and Byer surprised the medical community with their description of the high frequency of macroscopic lesions found in the coronary arteries of young American soldiers dead at the Corea war. (1) Nevertheless, in 1930 Zeek had previously concluded that arteriosclerosis might occur at any age. (2)

Later, Rapola and Pesonen reported the presence of lesions resembling preatherosclerotic changes in the main left coronary arteries of 14 dead newborns. These authors described intimal and medial changes with proliferation of smooth muscle cells (SMCs) that led to arterial wall thickening (3).

In spite of these findings, reports about lesions in coronary arteries of fetus and unweaned infants are uncommon and controversial. The level of blood lipids

in children determine, to a certain extent, the degree of coronary artery disease during adulthood and this observation has a relative consensus. (4)

Traditionally, lipid striates were considered earlier manifestations of atherosclerosis. Lipid striates are composed by extracellular lipids with some macrophages, and they are different from the lesions found in xantelasmas in cases of familiar hypercholesterolemia or in hypercholesterolemic rabbits. (5)

Nevertheless, initial atherosclerotic lesions in the coronary arteries are recognized since childhood for some authors (6-12) as an intimal proliferation of SMCs preceding the visible deposition of lipids that produces intimal thickening. (5, 13)

According to Virmani et al, (14) intimal thickening is an adaptive mechanism of most arteries which starts in the womb or after birth, and consists of a-

actin-positive SMCs surrounded by a matrix rich in proteoglycans. Macrophages are rarely detected. These lesions are more important at the sites of arterial bifurcation and they are considered the precursors of atherosclerotic lesions.

Our team described the scenario found in adult atherosclerotic lesions (15-18) and we have recently reported that intimal preatherosclerotic coronary disorders are detected during prenatal life and childhood. (19) We have found multifocal lesions in offsprings of smoking mothers; these lesions ranged from focal areas in fetus to young soft plaques in unweaned infants. These lesions showed proliferation of SMCs activated by gene *c-fos*, corroborated by a positive proliferating cellular nuclear antigen (PCNA). (19)

The aim of the present study was to assess the morphology of early atherosclerotic lesions in coronary arteries from sudden infant death syndrome (SIDS) victims in order to recognize the atherogenic mechanisms.

MATERIAL AND METHODS

We examined 52 victims of SIDS and 16 controls with non cardiac causes of infant death. SIDS victims were 1 day old to 1 year old (133.85 ± 100.02 days) and controls had an age of 164.19 ± 183.23 days. Non cardiac causes of death were as follows: anoxic encephalopathy ($n = 7$), head injury ($n = 3$), anencephaly ($n = 2$), pneumonitis ($n = 2$) and meningitis ($n = 2$).

In all the cases, no complications occurred during pregnancies, pregnant women did not report drug or alcohol consumption but maternal smoking during pregnancy was registered.

Autopsies were performed 6 to 18 hours after death. Hearts were fixed in 10% buffered formalin for 48 hours. The four major epicardial coronary arteries (the left main, the left anterior descending, the circumflex and the right coronary artery) were isolated and cut into 3-4 mm long cross sections. Each segment was sequentially labeled from the aortic ostium or from the origin of the left coronary artery. Pieces were dehydrated, embedded in paraffin and cut in serial sections. Cuts were stained with hematoxylin-eosin, Heidenhain's Azan stain, Alcian blue (pH 0.5 y 2.5) to de-

tect mucopolysaccharides, acetic orcein for elastic fibers and immunohistochemical studies.

Immunohistochemistry

Monoclonal antibodies were used for immunotyping of cells found in early lesions: lymphocytes (T cells, CD45RO, Biogenex; B-cells; CD20, Dako Co.; cytotoxic/suppressor, CD8, Dako Co.; helper/inductor, OPD4, Biogenex), macrophages (CD68, Dako Co.), endothelial cells (CD31, CD34, Biogenex, Factor VIII, Ylem-Milano) and SMCs (α -SM-Actin, MU128-UC 1A4 clone Biogenex).

The following detection systems were used: 1) biotin-estreptavidin- peroxidase (Biogenex, San Ramón, Ca), and 2) EPOS (Dakopatts, Carpinteria, Ca). Double labeling was achieved with EPOS: firstly, primary conjugated antibodies were labeled with peroxidase followed by a second labeling with biotin-estreptavidin-alkaline phosphatase. Peroxidase was detected with 3.3 diaminobenzidine and fast red was used to visualize the reactions with alkaline phosphatase; the former antigen stain was brown and the latter brilliant red. Negative control fractions were treated identically with an irrelevant antibody of the same isotype.

Morphometric study

All the histological slides were magnified and digitalized, and they were analyzed by a blinded observer. A Nikon Eclipse E400 microscope and a system for image analysis (Image Pro Plus for Windows, version 3) were used for histological and planimetric analysis. The luminal areas of the plaque were measured and the percentage of luminal stenosis was estimated. Table 1 and Figure 1 show the values of neointimal thickening, the perimeter of the internal elastic membrane, and the perimeter of the membrane affected by neointimal proliferation. A thickenss value greater than 25 mm was considered intimal thickening.

Intimal proliferation was defined according to the criteria of Angelini et al. (20) as muscular and elastic thickening, characterized by focal or diffuse proliferation of intimal SMCs with fragmentation or duplication of the internal membrane and deposition of collagen and elastin. Fibrous plaques were considered elevated focal lesions with proliferation of intimal SMCs blended in a matrix of connective tissue with lipids or without them.

Statistical analysis

The main histological results were compared with the two-tailed *t* test. Mann-Whitney test was used for non parametric statistical analysis. A *p* value < 0.05 was considered sta-

Table 1. Morphometry of coronary lesions

Neointimal thickening (μ m)	Affected perimeter of the IEM (μ m)	IEM Perimeter (μ m)	Affected perimeter of the IEM (%)	Area of neointimal proliferation (mm^2)	Luminal area (mm^2)	Stenosis %	
58.33 ± 17.8	915.6 ± 351.9	$2.008.3 \pm 585.9$	45.6%	0.029 ± 0.01	0.21 ± 0.1	13.8%	SDSI Intimal musculoelastic thickening without soft stenotic plaque
217.76 ± 26.4	910.1 ± 25.9	2092 ± 28.9	43.5%	0.10 ± 0.01	0.20 ± 0.02	50%	DIS Stenotic Soft plaque
84.95 ± 8.2	1131.6 ± 26.0	2570.5 ± 18.7	44.0%	0.052 ± 0.05	0.21 ± 0.1	24.8%	Single control compromised

IEM: Internal elastic membrane

tistically significant. Data were analyzed with a Statistic package version 7; values were expressed as mean \pm standard deviation.

RESULTS

Morphometric data are shown in Table 1. Preatherosclerotic lesions were found in 44.2% in the group SISD versus 6.3% in the control group ($p = 0.0062$).

Lesions ranged from focal plaques with mild myointimal thickening to a soft plaque which reduced the arterial lumen a 50% in one case. Left anterior descending coronary artery was compromised in all the cases.

Preatherosclerotic lesions were classified in two categories:

1. *Musculoelastic thickening*. These lesions showed mild to moderate focal myointimal thickening (Figure 2), sometimes located as an intimal cushion. The subendothelial connective tissue was infiltrated by SMCs, monocytes, rare lymphocytes and amorphous deposits. Smooth muscle cells lost polarity and gathered in pillars orientated perpendicular to the axis of the muscular layer infiltrating the subendothelium, with rupture of the internal elastic membrane (IEM) in most cases (Figures 3 and 4). Mucoid ground substance was also present. Macrophages were detected at the intimal edge penetrating the endothelium, but

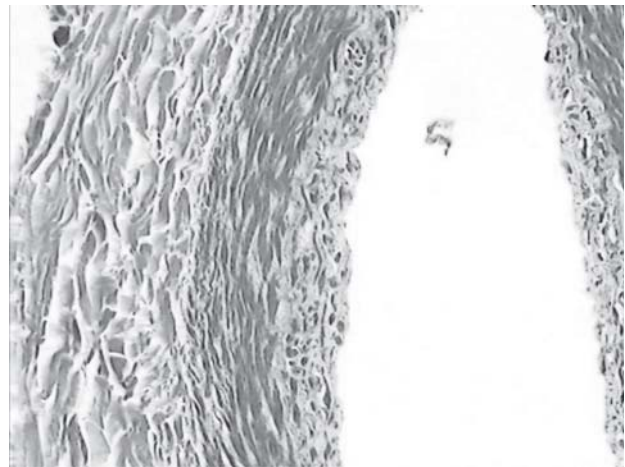


Fig. 2. Intima-media thickening with proliferation of SMC in the subendothelium in an epicardial artery from a patient with SISD. 40x lens objective, Azan's triple stain.

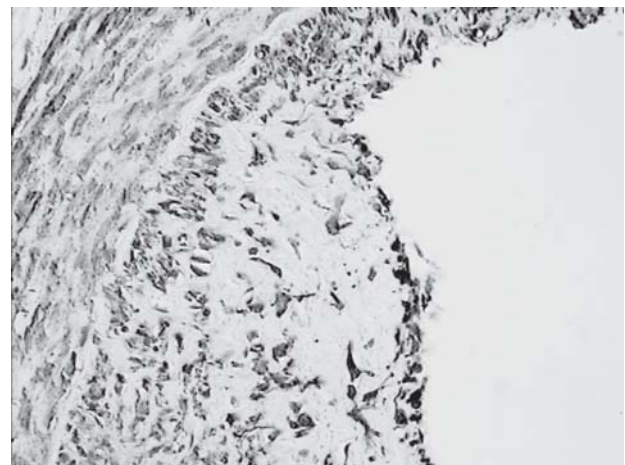


Fig. 3. Epicardial coronary artery with a soft plaque with subendothelial tissue infiltrated by proliferative SMCs α -actin-positive. 100x objective, anti-SMA.

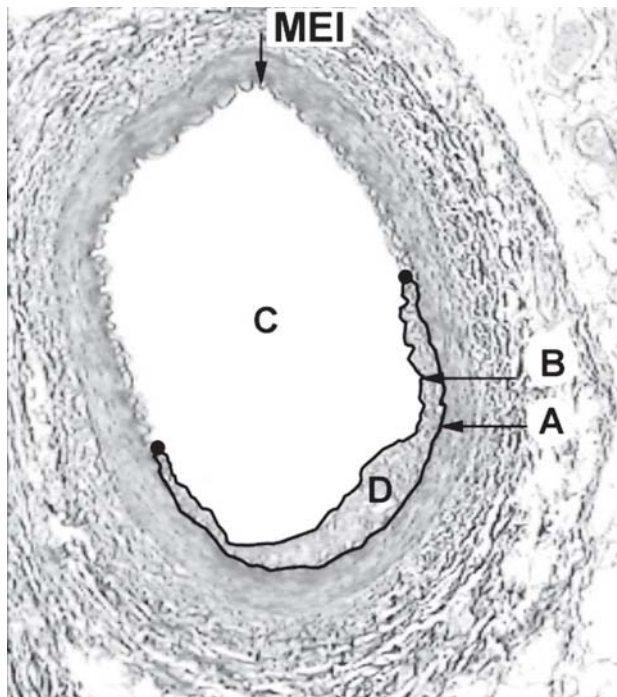


Fig. 1. Musculoelastic thickening of an epicardial coronary artery (left anterior descending coronary artery). IEM: Internal elastic membrane A: Affected perimeter of the IEM. Maximal distance A-B: Neointimal thickening. C: Luminal area. D: Area of neointimal proliferation. 40x lens objective, Mallory's triple stain.

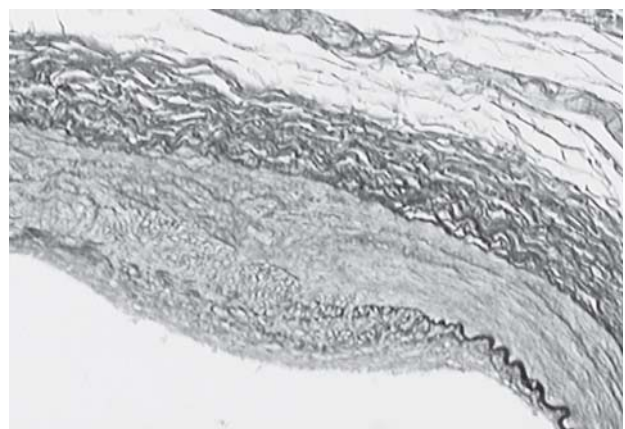


Fig. 4. Musculoelastic thickening of the left anterior descending coronary artery. A fragmented and duplicated internal elastic membrane is shown. 100x objective lens, orcein.

they were not observed inside the lesions. Angiogenesis was not observed, and few monocytes/foam cells and B lymphocytes were seen. The endothelium was intact and its surfaces were clean, without thrombus.

2. *Soft plaques* (Figure 3). These plaques showed high cellularity with infiltration of SMCs associated with mild infiltration of monocytes/foam cells, scarce lymphocytes and large amounts of mucoid ground substance. Internal elastic membrane and elastic fibers at the media were fragmented; the media was focally thinner, especially in areas with great proliferation. SMCs lost their polarity with the perpendicular axis of the media and migrated to the subendothelium. The endothelium was intact and no thrombi were detected.

Intermediate lesions, with components of both categories were frequent, thus they were considered musculoelastic thickening for the morphometric study (Table 1). In these cases, stenosis ranged from 9.5% to 24.6% ($13.8 \pm 5\%$) and the perimeter of the internal elastic membrane was affected in 45.6% of its length. Only one soft plaque (considered separately) had a reduction in the arterial lumen of 50%, an affected perimeter of 43.5% and an area of neointimal proliferation of 0.10 mm².

Of 68 cases, 31 mothers were smokers and 37 were non smokers. Lesions were present in 43% of cases (29/31) with a history of maternal smoking, while only 5/37 cases of non smoker mothers (7%) had lesions ($p < 0.0001$; Fisher's exact test).

DISCUSSION

This study demonstrates that early atherosclerotic lesions can be detected during childhood. These lesions range from muscular and elastic thickening of 24.6% of the lumen to a case of coronary stenosis of 50%. We were not able to establish a relationship between those lesions and SDIS; however, lesions were more frequent among SDIS group than in controls. It should be noted that the assessment of a causal relationship between SDIS and preatherosclerotic lesions was not the aim of this study; nevertheless, we took advantage of the material available from the autopsies as it is difficult to obtain these types of samples from unweaned infants. An unexpected finding was the greater incidence of these lesions in the SDIS compared to the control group (44.2% versus 6.3%; $p = 0.0062$). Mortality was not attributed to the presence of these lesions.

Classical studies have reported the presence of macrophages with lipids (foam cells) in segments of coronary arteries considered at danger in 33% of 6 month-old children. (21) During the following years, the accumulation of foam cells is less frequent in children than in unweaned infants. Then these lesions are present in 69% of adolescents between 12 and 15 years old. (12)

It has also been suggested that several changes take place at the intima as a response to an injury before foam cells start to accumulate. Chronodynamics of coronary heart disease was studied in transplanted human hearts; firstly, intimal hyperplasia and disruption of internal elastic membrane occur; secondly, SMCs migrate from the media to the thickened intima; and thirdly, lipid deposition leads to the development of an atheroma. (22)

This sequence of events is the precursor of atherosclerosis rather than benign changes in development and growth, as lipid deposition would never occur in the absence of these changes. (12)

These lesions, which might represent the missing link between normality and accumulation of foam cells and atheromas, were localized in areas at danger for atheromatosis, such as arterial bifurcations. (21)

These thick segments will subsequently contain small amounts of lipid drops and cellular detritus, in addition to foam cells. (21) Thick segments develop during fetal life (23) and are present in all newborns in variable ranges. (24) Thick segments are found in arterial bifurcations, in their proximities and in the ostia of small vessels, were the lesion is focal and eccentric. Diffuse intimal thickenings are found in other sites distant from vascular bifurcations. These thick segments are also known as adaptive intimal thickening, (14), intimal cushion, mucoid fibromuscular plaque, focal intimal hyperplasia, musculoelastic intimal thickening, etc. (24)

Some authors consider that thick segments are atherosclerotic lesions as they locate in sites where plaques develop in adults, such as the left anterior descending coronary artery, they are circumscribed and eccentric, and they project into the arterial lumen when they collapse after death. (21) These authors' findings are consistent with the musculoelastic intimal thickening reported in this study.

Therefore, although adaptive thickening is not a lesion in itself, it is a prerequisite for lipid trapping and accumulation which leads to atherogenesis. (21)

Anyway, in cases of severe hyperlipoproteinemia foam cells and lipids may accumulate and form plaques at any sites without previous adaptive thickening.

Foam cells have a half life of 6 months (21); thus, intimal changes seen during the first semester of fetal life might reflect maternal risk factors, such as smoking, lipid disorders, etc.

Clinical manifestations of atherosclerotic lesions might start as intimal thickenings. These lesions might stabilize and stop progressing. If risk factors change favorably, these lesions might experience regression and even disappear (21), or they might be functionally minimized through arterial remodeling and apoptosis. (25)

Intimal proliferation in the coronary arteries occurred in 95.3% of children between 1 and 5 years of

age, who died of non cardiovascular causes, and the proximal left anterior descending coronary artery was the most affected vessel. (20)

Focal Point: the Smooth Muscle Cell

SMCs have a leading role in early and established atherosclerosis; (16, 17) they represent half of the cellular components of chronic plaques and almost 90% in early plaques. (26) These cells have a phenotypic diversity and a rapid growth during embryonic development; nevertheless, they remain quiescent and differentiated in adults. Migration, proliferation and differentiation are pathological responses of these cells to different insults (such as smoking) which might contribute to the development of early atherosclerotic lesions and its progression, (19) possibly expressing genes which had been inactive during embryonic life. (27, 28)

We have recently reported that early atherosclerotic lesions in the coronary artery walls from fetus and weaned infants might be possibly related to maternal smoking. Some of these primary lesions showed proliferation of SMCs activated by gene c-fos, corroborated not only by a positive PCNA but also by apoptosis; the latter has been interpreted as a physiological attempt to prevent atherogenic evolution. (19) It is possible that cigarette smoke-related oxidants determine a sequence of biological events in the arterial walls. Firstly, oxidants might provoke endothelial dysfunction with no morphological disorders and they might immediately trigger gene c-fos from SMCs located at the media, promoting a proliferative process. (29) Theoretically, several molecules (PDGF, EGF, IL-1b, TNF α , etc.) might activate transcription factors such as nuclear factor-kappa B (NF- κ B) or proto-oncogenes such as c-fos and c-myc, which might regulate the expression of genes involved in the inflammatory/proliferative response of preatherosclerotic lesions. (30)

SMCs located at the media are contractile cells, with typical proteins as α -actin. When they migrate to the intima, SMCs show changes in their phenotype; they lose their contractile function and become synthesizer cells; in addition, actin expression changes from a to b, and the latter type has great proliferative capacity. (27, 28)

Moreover, a previous study reported that SMCs were PCNA-positive (75% of cases) and c-fos positive (40%) in a high percentage of unstable atherosclerotic plaques compared to stable plaques, (18) reflecting the importance of these cells in plaque instability.

Study Limitations

Specimens were taken from forensic autopsies, therefore it was not possible to perfuse the arteries and fix them at physiological pressures to preserve them from collapse and postmortem contraction. For the same reason, it was not possible to obtain a complete medical record of family history or genetic history in all cases.

CONCLUSION

Preatherosclerotic lesions develop early in SIDS victims. Smooth muscle cells are fundamental in its genesis. Proliferation of SMCs and their migration to the intima is the touchstone of the process. Further studies should be performed to elucidate the relationship between atherosclerosis and SIDS.

RESUMEN

Lesiones ateroscleróticas tempranas en el síndrome de muerte súbita infantil

Objetivo

El presente estudio se llevó a cabo con el propósito de caracterizar morfológicamente lesiones ateroscleróticas precoces en arterias coronarias de víctimas del "síndrome de muerte súbita infantil" (SMSI) para conocer los mecanismos aterogénicos.

Material y métodos

Se efectuó el examen de 52 víctimas de SMSI y de 16 casos controles fallecidos de causas conocidas. Las principales arterias coronarias se cortaron serialmente y se tiñeron con hematoxilina-eosina, Azán, azul alciano, orceína acética, CD68, CD34 y α -SM-actina y se realizó histomorfometría de las lesiones.

Resultados

Se encontraron lesiones preateroscleróticas en el 44,2% del grupo SMSI (23/52) y en sólo el 6,3% del grupo control (1/16) ($p = 0,0062$). Las células musculares lisas perdieron la polaridad, infiltrándose en el subendotelio, en gran parte de los casos con rotura de la membrana elástica interna (MEI). No se observó neoangiogénesis. En el grupo SMSI con engrosamiento musculoelástico intimal, el espesor neointimal fue de $58,3 \pm 17,8$ μ m, el perímetro de la MEI afectado fue del 45,6%, el área de proliferación neointimal fue de $0,03 \pm 0,01$ mm^2 y el área luminal fue de $0,21 \pm 0,1$ mm^2 con $13,8\% \pm 5\%$ de estenosis luminal.

Conclusiones

Las lesiones preateroscleróticas se desarrollan temprano en víctimas del SMSI y son significativamente más frecuentes que en los controles. Las células musculares lisas tienen un papel fundamental en su génesis.

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Palabras clave > Aterosclerosis - Muerte súbita del lactante - Vasos coronarios

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