Early atherosclerotic lesions and post-mortem serum cholesterol level in a group of Colombian children

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Introduction: Atherosclerosis is an asymptomatic chronic disease, which begins at early age and is difficult to detect during this stage. Prospective studies suggest a causal relationship between total serum cholesterol levels during childhood and early adolescence and the development of cardiovascular diseases in adulthood.

Objective: The aim of this study was to evaluate the prevalence of early atherosclerotic lesions in the aorta artery of children and its relationship with post-mortem serum cholesterol levels.

Material and methods: Post-mortem samples of blood and aorta were taken from 43 subjects aged less than 17 years old. Histopathological analysis (intimal thickening and inflammatory infiltrates) of the thoracic aorta and measurement of total serum cholesterol were performed.

Results: The analysis showed thickening of the intima and lymphocyte infiltrates in 93% of children, and macrophage infiltrates in 79.1% of cases. A relationship between the highest terciles of total serum cholesterol levels and the presence of multiple lesions in the aorta wall was found (p<0.05).

Conclusion: This group of children had a high prevalence of early inflammatory atherosclerotic lesions positively related with serum cholesterol levels. To our knowledge this study represents the first report of a relationship between post-mortem total serum cholesterol levels and pathological findings of macrophages and lymphocytes infiltrates in the aorta wall.

Key words: Atherosclerosis, child, adolescent, cholesterol, post-mortem changes, aorta.

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Lesiones ateroscleróticas tempranas y nivel de colesterol post mótem en un grupo de niños colombianos

Introducción. La aterosclerosis es una enfermedad crónica asintomática que se inicia a edad temprana y es de difícil detección en esta etapa. Los estudios prospectivos sugieren una relación causal entre el nivel de colesterol sérico total en la niñez y la adolescencia, y el desarrollo de enfermedades cardiovasculares en la adultez.

Objetivo. El objetivo de este estudio fue evaluar, post mótem, la prevalencia de lesiones ateroscleróticas tempranas en la arteria aorta de niños y su relación con los niveles de colesterol.

Materiales y métodos. Se tomaron muestras de sangre y de aorta de 43 sujetos con edades menores de 17 años. Se hizo el análisis histopatológico (engrosamiento de la íntima e infiltrado inflamatorio) de la aorta torácica y la medición de colesterol en suero.

Resultados. El análisis mostró engrosamiento de la íntima e infiltrado linfocitario en 93 % de los niños, e infiltrado de macrófagos en 79,1 %. Se encontró relación entre los terciles superiores de colesterol sérico y la presencia de múltiples lesiones en la pared de la aorta (p<0,05).

Conclusión. Este grupo de niños presentó una alta prevalencia de lesiones ateroscleróticas inflamatorias tempranas relacionada positivamente con niveles de colesterol sérico. Para nuestro conocimiento, este estudio representa el primer reporte de la relación entre el colesterol sérico...
y los hallazgos histopatológicos de infiltrado de macrófagos y linfocitos en la pared de la aorta, post mórtem.

**Palabras clave:** aterosclerosis, niño, adolescente, colesterol, cambios post mortem, aorta.

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Atherosclerosis is an asymptomatic chronic process, which begins at early age and progresses into adulthood (1,2). Post-mortem studies in children and fetuses have reported the presence of atherosclerotic lesions (3). These findings have been confirmed by the presence of fatty streaks in aortas of children below three years old and in coronary arteries of children between 10 and 14 years of age (1-3). Histological observations have shown that the lesions developed from six months of age and may progress until forming fibrous plaques in coronary arteries (4).

Atherosclerosis is difficult to detect during childhood because the lesions, in case of being present, are not easily observable, and conventional risk factors (e.g., sedentary lifestyle, obesity, smoking, alcoholism), which have a harmful effect on vascular endothelium, are still not evident. However, prospective studies suggest a causal relationship of serum cholesterol levels during childhood and early adolescence with coronary disease during adulthood (5).

Total serum cholesterol is considered a good post-mortem biomarker because of its stability and sensitivity as a cardiovascular risk factor (6). Some autopsy studies have showed that early atherosclerosis is related with lipid fractions like high serum total cholesterol levels, LDL cholesterol, and low HDL cholesterol (7-8). However, reports of these associations in Latin American populations are unknown. Likewise, there are no evaluation reports of the relationship between post-mortem serum cholesterol and lesions of cellular infiltration (macrophages and lymphocytes). Therefore, the aim of this study was to evaluate the post-mortem prevalence of early atherosclerotic lesions (intimal thickening and presence of lymphocytes and macrophages) in the thoracic aorta and its relationship with serum cholesterol in a group of Colombian children.

**Materials and methods**

**Subjects**

This study analyzed 43 samples of thoracic aorta and blood of autopsies of children done at the Instituto Nacional de Medicina Legal y Ciencias Forenses in the city of Cali. We included autopsies of children less than 17 years old, with accidental or violent cause of death and without severe damage to the cardiac cavity. Parents or relatives gave a written consent. The research protocol was approved by the Human Ethics Committee in the Facultad de Salud at Universidad del Valle in Cali, Colombia.

**Histopathology**

The thoracic aortas were removed from a 2 cm proximal point to the ligamentum arteriosum to a 2 cm distal point to the iliac bifurcation. The samples were incubated in 10% formalin for 24 hours. Then, they were dehydrated and embedded in paraffin. Tissue sections (6 μm) were cut and stained with hematoxylin-eosin (9) and were examined by a certified pathologist. Microscopic evaluation considered intimal thickening, as well as the presence of inflammatory cells (macrophages, lymphocytes) using a visual analogue scale (10). The presence of macrophages and lymphocytes was defined as having a moderate and abundant amount of these cells, while a low amount or nothing was considered as absence.

**Post-mortem serum cholesterol**

The blood samples were obtained by cardiac puncture with a 10 ml syringe and transferred into a tube without any anticoagulant. The sample was centrifuged for 10 minutes at 4,000 rpm; the serum was extracted and stored at -20°C. The serum cholesterol was measured by the Abell-Kendall method recommended by the Center for Disease Control (CDC, Atlanta GA), modified with previous standardization (11). For processing, 0.5 ml of serum were mixed with 5 ml of KOH at 33% ethanol and incubated at 50°C for 1 hour. Afterwards, 10 ml of n-hexane were added and centrifuged for 5 minutes at 2,500 rpm. The n-hexane layer was desiccated at 62°C and the precipitate was suspended again in 6 ml of the Liebermann-Burchard stable reactive (12). The absorbance was read at 620 nm (UV-1700...
SHIMADZU). As a standard, we used cholesterol (Sigma, HPLC grade), and for quality control, we used clinical biochemistry control serums (Biosystems Spain).

**Statistical analysis**

Data analysis was performed using SPSS software (version 11.0). Post-mortem serum cholesterol was divided in terciles. The proportions difference was estimated by $\chi^2$ and Fisher’s exact tests, and the relationship between age and post-mortem serum cholesterol by Spearman correlation. A p value less than 0.05 was considered significant, a p value between 0.05 and 0.1 was considered as marginal significance.

**Results**

Characteristics of the subjects are shown in table 1. Almost the totality of the group was male and 84.4% were adolescents. The mean total serum cholesterol of the subjects (133 mg/dl) was in the normality range. Prevalence of early atherosclerotic lesions, i.e. intimal thickening and presence of abundant lymphocytes and macrophages was found in almost all of the subjects. In 75% of the subjects, intimal thickening, lymphocytes and macrophages were found concomitantly.

We found a higher prevalence of atherosclerotic lesions in the second and third tercile of cholesterol (figure 1). By specific lesion, the presence of macrophages was higher in terciles 3 (156-257 mg/dl) and 2 (109-156 mg/dl) in relation to

Table 1. Population characteristics

<table>
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<tr>
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<th>n (%)</th>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
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<tr>
<td>Male</td>
<td>39 (90.7)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (9.3)</td>
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<tr>
<td><strong>Age group</strong></td>
<td></td>
</tr>
<tr>
<td>≤5 years old</td>
<td>1 (2.3)</td>
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<tr>
<td>&gt;5 and &lt;12 years old</td>
<td>4 (9.3)</td>
</tr>
<tr>
<td>≥12 years old</td>
<td>38 (84.4)</td>
</tr>
<tr>
<td><strong>Early atherosclerotic lesions</strong></td>
<td></td>
</tr>
<tr>
<td>Intimal thickening</td>
<td>40 (93.0)</td>
</tr>
<tr>
<td>Presence of lymphocytes</td>
<td>40 (93.0)</td>
</tr>
<tr>
<td>Presence of macrophages</td>
<td>34 (79.1)</td>
</tr>
<tr>
<td>All three early atherosclerotic lesions</td>
<td>32 (74.4)</td>
</tr>
<tr>
<td><strong>Serum total cholesterol (mg/dL)</strong></td>
<td>134±45</td>
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1Mean ± standard deviation

**Figure 1.** Prevalence of early atherosclerotic lesions by terciles (T) of serum cholesterol. (A) Intimal thickening, B) presence of macrophages, C) presence of lymphocytes, and D) all three early atherosclerotic lesions. Differences between T1 (53-108 mg/dl), T2 (109-156 mg/dl) and T3 (156-257 mg/dl) are shown.
cholesterol tertile 1 (53-108 mg/dl) with marginal significances (p=0.089 and p=0.052, respectively) (figure 1B). The infiltrate lymphocytes and intimal thickening were significantly higher in tertile 3 in relation to tertile 1 (p=0.089 (marginal) and p=0.09, respectively) (figures 1A and 1C). While the prevalence of all atherosclerotic lesions evaluated was also significantly higher in the second and third terciles of serum cholesterol vs first tertile (figure 1D), differences in the prevalence of lesions between the second and the third terciles of serum cholesterol were not observed.

Age showed a weak correlation with postmortem serum cholesterol levels with marginal significance (rho=0.278, p=0.071), but it was not associated with any early atherosclerotic lesions (data not shown).

Discussion
In this study, we found an unexpected high prevalence of early atherosclerotic post-mortem lesions in a group of Colombian children, and this prevalence was associated to a higher serum cholesterol level. Post-mortem studies have revealed that the atherosclerosis process starts from childhood (2-6), where the earliest vascular finding is the fatty streak, as a result of the accumulation of cholesterol inside the macrophages and other lipids in the intimal-medial of the artery. This lesion could progress to a chronic inflammatory state because the continuous infiltration and accumulation of macrophages and lymphocytes, accompanied by the proliferation of smooth muscle cells, lead to intimal-medial thickening.

We found correlations with marginal significance between elevated levels of post-mortem serum cholesterol and the degree of infiltration in the aorta vascular wall by macrophages and lymphocytes, which are compatible with a chronic inflammatory state, characteristic of the atherosclerotic process. This finding agrees with other studies that reported a relationship between serum cholesterol, endothelial dysfunction, and intimal-medial thickness (13,14).

Our study found a mean post-mortem serum cholesterol of 133 mg/dl, lower than that reported in a population of Colombian children of the same age range (168 mg/dl) (15). A possible explanation for this difference is the fact that most of the subjects we studied (85%) were in the age of puberty development, a stage that has been associated to diminished serum cholesterol (16). Likewise, most of the subjects (76.7%) had levels of cholesterol <170 mg/dl, the cut-off point recommended by the United States National Cholesterol Education Program (NCEP) for pediatric populations (17,18).

Regarding lesions in the aorta, we observed an association between high tertile serum cholesterol with the presence of all histopathological alterations evaluated. The Bogalusa Heart study reported an association between total cholesterol and the presence of fatty streaks in the aorta and the coronary arteries (19). These findings show a possible early relationship between cholesterol levels and the chronic inflammatory process in the artery wall, even when the cholesterol levels are in the normal range.

To our knowledge this study represents the first report of a relationship between post-mortem serum cholesterol and pathologic findings of macrophages and lymphocytes infiltration in aorta wall, and the first evaluation precedent of an association between post-mortem serum cholesterol and early atherosclerotic lesions in Latin American children. Although our sample size was small, this limitation can be lessened by the high prevalence of incipient atherosclerotic lesions found in the children group.

The findings of this study suggest the need to implement preventive measures in pediatric populations to reduce the morbimortality related with inflammatory atherosclerotic process and also the importance to measure cholesterol in childhood as a possible predictor of early atherosclerosis and provide rationale for recent recommendations for pediatric lipid screening (20,21) and, furthermore, the modification of unhealthy life styles in early ages in order to reduce the serum cholesterol levels(22).

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Conflict of interest
The authors declare no conflict of interest

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