Histological demonstration of early atherosclerosis by "oil-red-O" staining in neonatal cadavers

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Since atherosclerosis is one of the main contributing factors in mesenteric ischemia which is a real dilemma to diagnose and to treat as a cause of acute abdomen with no improvement to deal with for centuries, the analysis of atherosclerosis of the abdominal aorta and its branches with oil red O lipid staining in neonatal cadavers was aimed. According to the unchanged motto still shared unfortunately by almost all physicians including surgeons even of our era, occlusion of the mesenteric vessels is apt to be regarded as one of those conditions of which the diagnosis is impossible, the prognosis hopeless and the treatment almost useless. Early diagnosis and aggressive treatment can only be possible in the future with understanding the pathophysiology of mesenteric ischemia and its contributing factor namely "atherosclerosis" which is the most important underlying etiology. Prognosis of mesenteric ischemia did not improve despite progresses in technology for decades. Acute abdomen caused by mesenteric ischemia has a mortality rate still ranging between 70-90% despite new technologies developed for its diagnosis and its treatment.The incidence of mesenteric ischemia increases as well as coronary artery diseases not only because of the increment of the elderly population. Therefore, human anatomic studies can help the clinicians and 22 newborn human cadavers were dissected to demonstrate atherosclerosis with "oil-red O lipid staining" in the abdominal aorta and its main branches to localize the lesions which are claimed to be important for the etiology of mesenteric ischemia. Although the signs and symptoms of atherosclerosis can not be encountered untill late adulthood, it is widely known that atherosclerotic process begins earlier in life. Atherosclerotic process begins in the neonatal life according to our hypothesis, for which we aimed to find an evidence to support. Although the main problems involve coronary arteries of heart and cerebral arteries, aorta is the first vessels to be involved by atherosclerosis. Therefore we stained aorta and its main branches. Large vessels are more sensitive to low shear stress. Therefore a. mesenterica superior (AMS) is is more sensitive to atherosclerosis when compared with a. mesenterica inferior (AMI). Branching points with an angle less than 90 degrees are more sensitive to atherosclerosis.Therefore AMS is more sensitive to atherosclerosis when compared with truncus coeliacus. Bending points of vessels, lateral walls of bifurcations, posterior walls of the main vessels just opposite to branching points are also sensitive to atherosclerosis [1,2].

15 male and 7 female neonatal cadavers were dissected in our study in the laboratory of Marmara University Medical Faculty, Anatomy Department. Crosssections at 5 aortic (A) levels (A 1-5) and at its large branches in addition to AMS and AMI. A1: Just superior of Truncus coeliacus, A2: Between Truncus coeliacus and AMS, A3: Just inferior of AMS, A4: Just superior of AMI, A5: Just inferior of AMI. AMS, AMI, truncus coeliacus, arteria

hepatica communis, arteria lienalis, arteria gastrica sinistra: All these vessels were cut at their origins. Equipment for histological demonstration were Formaldehyde (%10), % 30g Sucrose solution, % 0.7g Oil red O solution (Merck Darmstadt/Germany), %100 Propylene glycole solution, %1g gelatin solution: (Merck-Gelatin for microbiology-104070), Hematoxilene (JT Baker-3873), Neutral buffer PBS (Phosphate Buffered Saline) solution, PH: 7.2 liquid glycerol gelatin (Merck-109242 Kaiser's Glycerol Gelatine for Microscopy), Freezing medium agent, cryomatrix (Bio Optica-059801), Qualitative filter paper (Whatman No: 1002-125), Thermo Fisher Scientific Thermoshandon MX35 Premier Plus 3052835, 50 blades. Vessels were freezed at -21 grade Celcius on Cryomicrotome (frozen section) and were cut with a thickness of 10 μm and these cross-sections were stained by oil red O. In our study, we chose abdominal aorta and its branches since we aimed to study the atherosclerotic aspect of mesenteric ischemia where aorta (especially abdominal aorta) is the first vessel to be effected by atherosclerosis (even before coronary and cerebral arteries). Atherosclerosis is a systemic disease and is diffuse in all vessels.

Oil Red O staining was overall positive, either fatty streak or atheroma, in 9 cadavers (9/22, 41%). Results in vessels other than aorta were positive in 8 cadavers such as positive staining of arteria lienalis in 3 cadavers (cadaver no: 16, 18, 20), positive staining of truncus coeliacus in 2 cadavers (cadaver no: 17, 21), positive staining of arteria mesenterica superior in 2 cadavers (cadaver no: 3, 13), positive staining of arteria mesenterica inferior in 1 cadaver (cadaver no: 5). When aorta cross sections were evaluated, lipid staining gave positive result only in one cadaver in whom no stains were detected in smaller vessels (cadaver no: 11). All 8 cadavers with positive lipid stain other than aorta had lipid staining at aortic specimens at different levels, although not all sections. Evaluation of aortic sections: Oil red O staining was detected mostly at A-2 and A-3 levels (just superior and inferior to a. mesenterica superior). Documentation of the levels of the 9 Aorta overall (with positive lipid stain) were: A-3 in 7 cadavers, A-2 in 4 cadavers, A-1 in 2 cadavers. A-5 in 2 cadavers, and A-4 in 1 cadaver.

Atherosclerosis begins in the neonatal period. We concluded that aortic lipid staining was not similar to adult type distribution in our series. In adults, infrarenal aorta (A-4 ve A-5) is the most sensitive part of aorta. In neonates, adult type atherosclerosis should not be expected. According to the literature, lipid deposition is mostly encountered at distal aorta around the origin of arteria mesenterica inferior (especially at the posterior aortic wall) in adult cadavers, since this area empties in diastole, which causes turbulent, retrograde and instabile flows. However, we detected more lipid deposition at superior levels since we did not study on adult cadavers. Therefore we can conclude that bifurcation atherosclerosis is a disease of old age, rather than of the neonatal period. Adaptive degenerative changes begin with increasing age. Expansive remodeling, as a response to atherosclerosis, to increase the aortic lumen is an adaptive change but it is also a “wolf in sheep’s clothes” entity since it leads to aneurysms. Underlying mechanisms are as follows: 1- Lower levels of aorta abdominalis have relatively less amount of vasovasorum, hence the wall of the distal aorta is relatively less well nourished. 2-There is a standard blood flow throughout the whole day in the thoracic aorta and upper levels of aorta abdominalis, because skeletal activity is not present in these levels and therefore changing in blood flow during muscle activity is not encountered. 3-Decreased physical activity with increasing age influences the lower levels of aorta and flow volume decreases where blood flow slows. 4-Infrarenal aorta dilates with increasing age which causes the process of atherosclerosis to ascend in the aorta [1- 3].

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