ORIGINAL ARTICLE / KLİNİK ÇALIŞMA

# Assessment of the relationship among breast milk intake, birth pattern, antibiotic use in infancy, and premature atherosclerosis

## Anne sütü alımı, doğum şekli ve süt çocukluğu döneminde antibiyotik kullanımının prematür ateroskleroz ile ilişkisinin değerlendirilmesi

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## ABSTRACT

***Objective:*** Previous studies have shown cesarean section (C-section) and antibiotic use in the infantile period lead to chronic diseases in later life. It is also known that breastfeeding, which forms the basal system of immunity, is a protector in neonates. In this study, we aimed to investigate the association between breastfeeding, antibiotic use, C-section, and premature atherosclerosis.

***Methods:*** A total of 100 patients who underwent coronary angiography and had stenosis in at least 1 epicardial vessel and 100 controls with normal coronaries were included in the study. In addition to traditional risk factors, type of delivery, breast milk intake and duration, and antibiotic use and frequency were evaluated for each participant. Lipid profile was added to the study procedure. Angiographic images of the study groups were examined to calculate the Gensini score.

***Results:*** Smoking, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and family history were different between the groups. In the control group, 3 were born via C-section, whereas 26 were born via C-section in the atherosclerosis group (p<0.001). Breast milk intake and duration was also significantly higher in the control group (p=0.018). Antibiotic use was less in the control group, but there was no statistically significant difference (p=0.099). In multivariate logistic regression analysis, diabetes mellitus, smoking, and C-section were predictors of atherosclerosis (p=0.036, p=0.001, and p=0.003, respectively). In receiver operating characteristics curve analysis, the ability of C-section to predict premature atherosclerosis was superior to diabetes but not to smoking (area under curve, 0.607; p=0.023).

***Conclusion:*** Mode of delivery and breast milk intake should be evaluated and considered among the risk factors of premature atherosclerosis.

## ÖZET

***Amaç:*** Önceki çalışmalarda sezaryen ve infantil dönemde antibiyotik kullanımının daha sonra kronik hastalıklara yol açabileceği gösterilmiştir. Ayrıca, bağışıklığın bazal sistemini oluşturan anne sütünün de yenidoğanlarda bir koruyucu olduğu bilinmektedir. Çalışmamızın amacı, anne sütü alımı ve süresi, doğum şekli, infantil dönemde antibiyotik kullanımı ile prematür ateroskleroz arasındaki ilişkiyi belirlemektir.

***Yöntemler:*** Koroner anjiyografi yapılan ve en az bir epikardiyal damarda darlığı olan 100 hasta ve normal koroner saptanan 100 kontrol hastası çalışmaya dahil edildi. Geleneksel risk faktörlerine ek olarak her katılımcı için doğum şekli, anne sütü alımı ve süresi, antibiyotik kullanımı ve sıklığı değerlendirildi. 12 saat açlık sonrası alınan venöz kan örnekleri incelenerek hastaların kolesterol değerleri kaydedildi. Gensini skorunu hesaplamak için çalışma grubunun anjiyografik görüntüleri incelendi.

***Bulgular:*** Sigara kullanımı, yüksek yoğunluklu lipoprotein kolesterol, düşük yoğunluklu lipoprotein kolesterol ve aile öyküsü açısından gruplar arasında istatistiksel olarak anlamlı farklılıklar bulundu. Kontrol grubundakilerden üçü, ateroskleroz grubundakilerden 26'sı sezaryen ile doğmuştu (p<0.001). Anne sütü alımı ve süresi de kontrol grubunda anlamlı olarak yüksekti (p=0.018). Kontrol grubunda antibiyotik kullanımı daha azdı, ancak iki grup arasında istatistiksel olarak anlamlı bir fark yoktu (p=0.099). Çok değişkenli lojistik regresyon analizinde; diabetes mellitus, sigara ve sezaryen aterosklerozun belirleyicileri olarak bulundu (p=0.036, p=0.001 ve p=0.003, sırasıyla). ROC analizinde sezaryen prematür aterosklerozu öngörücü rolü diyabetten fazla sigara kullanımından azdı (eğri altındaki alan: 0.607, p=0.023).

***Sonuç:*** Doğum şekli ve anne sütü alımı erken ateroskleroz risk faktörleri arasında değerlendirilmeli ve dikkate alınmalıdır.

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The newborn period is prone to inflammatory changes that could affect lifetime immune response leading to atherosclerotic cardiovascular disease in adulthood.[1] McDade et al.[2] have pointed out that the C-reactive protein levels in the samples taken in adulthood of individuals breastfed for 3 months or longer were significantly lower than those in the samples of individuals fed with formulas and suggested that breastfeeding may have relevant effects on reducing chronic inflammation and lowering the risks for cardiovascular diseases. Studies have shown that oxidative stress development linked with chronic inflammation is strongly associated with impaired immune response.[3] The contact with the vaginal flora at birth is essential for the development of a neonate's immunity. The remnants of the vaginal flora contacted by the skin and organs' epithelium during vaginal delivery may last up to 2 years, stimulating the foundation of the gut microbiota and a healthy immune system.[4,5] Any disturbance in the microbiota during the first 2 years of life bears critical importance. The exposure to antibiotics in the first years of life may result in dysbiota or dysbiosis. Studies have shown that dysbiosis was associated with chronic inflammatory diseases.[6]

In this study, we aimed to investigate the relationship between cesarean section (C-section), breast milk intake, antibiotic use, and premature atherosclerosis.

## METHODS

### Study population

The retrospective and cross-sectional study was conducted at Adana City Training and Research Hospital and included the files of 200 patients who underwent coronary angiography (CAG) between May 2018 and May 2019 because of ischemia or suspected ischemia. The study was approved by the Clinical Research Ethics Committee of Adana City Training and Research Hospital with number 664 on 08/01/2020, and the study was conducted according to the principles of the Declaration of Helsinki.

The patients were dichotomized into study and control groups depending on whether they had 1 or more stenotic epicardial vessels or none, on the basis of the CAG findings. Patients with stenosis in at least 1 epicardial vessel in CAG (n=100) were classified as the atherosclerosis group, and patients with normal coronary arteries (n=100) were the control group. Patients below 18 years of age and above 40 years, who had chronic cerebrovascular, kidney, liver, lung, inflammatory diseases, and sleep apnea were excluded. The patients with hypertension (HT) and diabetes mellitus (DM) were not excluded because of the close association of the nature of the diseases and the chronic inflammation. The flow diagram of the study is presented in Figure 1.

**Abbreviations:**

| | |
|---|---|
| *CAD* | *Coronary artery disease* |
| *CAG* | *Coronary angiography* |
| *CI* | *Confidence interval* |
| *C-section* | *Cesarean section* |
| *DM* | *Diabetes mellitus* |
| *HDL-C* | *High-density lipoprotein cholesterol* |
| *HT* | *Hypertension* |
| *LDL-C* | *Low-density lipoprotein cholesterol* |
| *PCI* | *percutaneously intervened* |
| *ROC* | *Receiver operating characteristics* |

HT was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or patient taking antihypertensive medication. DM was defined as fasting serum glucose ≥126 mg/dL, hemoglobin A1c ≥6.5%, or patient taking blood glucose lowering agents.

The mode of delivery, C-section or vaginal, breast milk intake and duration, and antibiotic use in the first 2 years of life were recorded for each patient. Incomplete or uncertain information was excluded from the analysis.

### CAG and Gensini score

All patients were catheterized percutaneously with the standard Judkins technique by cardiologists who were blinded to the study data.[7,8] The severity of the coronary disease was determined by the Gensini score for the study group. A Gensini score of 1 to 20 was considered as mild coronary atherosclerosis, and a score above 20 was considered severe coronary atherosclerosis.[9,10]

The data collected from the files included the baseline blood count and routine blood chemistry analyses of the samples taken in the week of CAG. Serum lipid levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured using xylidine blue with an end-point colorimetric method. In addition to the demographics, a history of smoking was questioned and recorded for each participant.

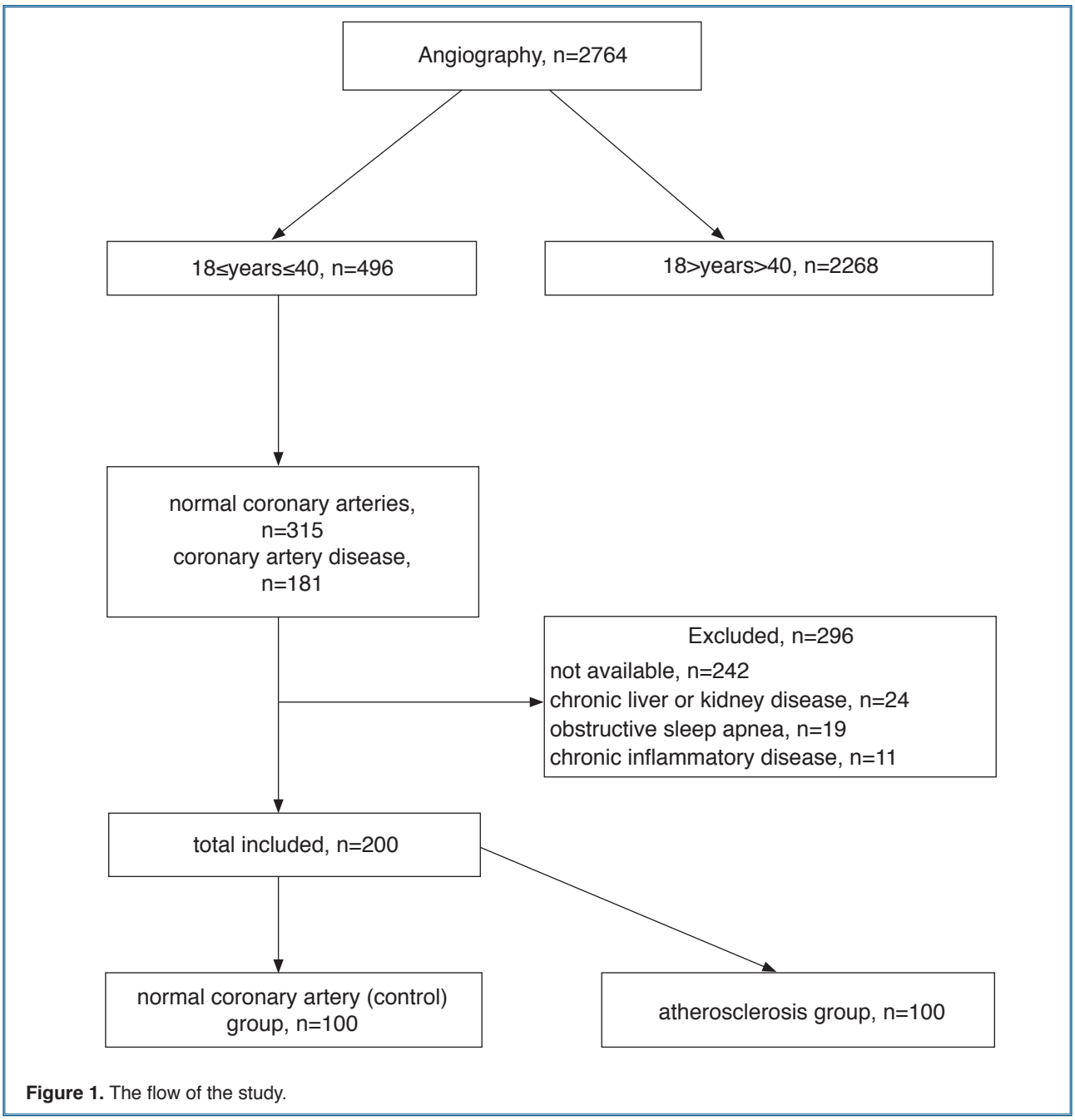



**Figure 1.** The flow of the study.

## Statistical analysis

All statistical analyses were performed with SPSS version 17 (SPSS Inc.; Chicago, IL, USA) and MedCalc (MedCalc Software Ltd; Ostend, Belgium) for Windows (Microsoft Corporation, Redmond, Washington, USA). The continuous variables were expressed as mean±standard deviation (mean±SD) or median (interquartile range), and the categorical variables were expressed as numbers and percentages. The comparisons of the continuous variables between groups were performed using the independent samples t test and Mann-Whitney U test, as appropriate, and the categorical variables using the $\chi^2$ test or Fisher's exact test. Whether continuous variables had normal distribution was analyzed with the Kolmogorov-Smirnov test. The relationship among parameters with normal distribution were assessed using Pearson's correlation analysis, and the Spearman rank correlation coefficient was used for variables with non-normal distribution. The Hosmer-Lemeshow test was used for goodness of fit for logistic regression

**Table 1. Baseline characteristics and laboratory findings of the groups**

| | | Atherosclerosis group (n=100) (mean±SD, n) | Control group (n=100) (mean± SD, n) | *p* |
|---|---|---|---|---|
| Age, years | | 37.1±2.6 | 36.8±3.5 | 0.548 |
| Sex, female/male, n | | 30/70 | 41/59 | 0.104 |
| DM, n | | 13 | 8 | 0.192 |
| HT, n | | 4 | 10 | 0.122 |
| Smoking, n | | 75 | 44 | <0.001* |
| Family history, n | | 63 | 41 | 0.002* |
| Mode of delivery, N/C-section, n | | 74/26 | 97/3 | <0.001* |
| Breastfeeding, n | Never | 17 | 9 | 0.018* |
| | ≤6 months | 38 | 29 | |
| | 6-12 months | 31 | 31 | |
| | ≥12 months | 14 | 31 | |
| Antibiotic exposure, n | Never | 67 | 78 | 0.099 |
| | Rarely (once or twice per year) | 20 | 17 | |
| | Often (more than twice per year) | 13 | 5 | |
| BMI, kg/m$^2$ | | 27.4±4.5 | 28±5,4 | 0.440 |
| Triglyceride, mg/dL | | 234.7±139.2 | 199.7±113.9 | 0.091 |
| LDL-C, mg/dL | | 145.0±62.0 | 126.8±28.8 | 0.019* |
| HDL-C, mg/dL | | 41.3±11.2 | 44.7±9.9 | 0.048* |

*Statistically significant.
BMI: body mass index; C-section: cesarean section; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; N: vaginal delivery; SD: standard deviation; DM: diabetes mellitus; HT: hypertension.

models to analyze univariate, and multivariate logistic regression analyses were performed to assess the relationship between coronary artery disease (CAD) and mode of delivery, human breast milk intake, antibiotic exposure, and other risks. Risk factors with a p≤0.25 in univariate analysis were included in the multivariate analysis. Receiver operating characteristics (ROC) curve analysis was used to analyze the predictive ability of variables. The results were expressed as relative risk and 95% confidence interval (CI). A p value of less than 0.05 was considered statistically significant.

## RESULTS

The mean age of the patients was 37.0±3.1 years (35.5% female). The baseline characteristics of the patients are summarized in Table 1. The smoking rate and the rate of CAD in first-degree family members of the atherosclerosis group were significantly higher than that of the control group (p<0.05 for both groups).

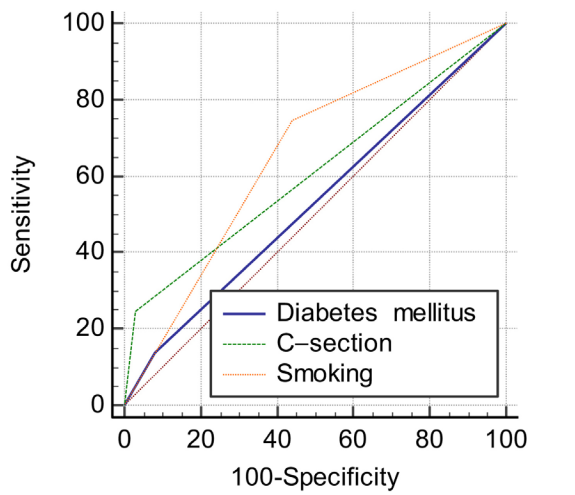
The rate of cesarean delivery at birth was statistically significantly higher in the atherosclerosis group than in the control group (26% [n=26] and 3% [n=3]; p<0.001, respectively). In the study group, 17% of patients were not fed breast milk, 38% were breastfed for less than 6 months, 31% between 6 and 12 months, and 14% up to 2 years. In the control group, the rates were 9%, 29%, 31%, and 31%, respectively (p=0.018). There was no significant differences between the groups in terms of antibiotic use in the first 2 years of life, albeit the higher rate in the control group (p=0.099). The mean LDL-C was significantly higher in the atherosclerosis group than in the control group, and the mean HDL-C was significantly lower (p<0.05, both groups).

The univariate and multivariate analysis are summarized in Table 2. The multivariate regression analysis showed that C-section (95% CI: 0.025-0.473; p=0.003), smoking (95% CI: 0.104-0.556; p=0.001), and DM (95% CI: 0.046-0.900; p=0.036) were independent predictors of premature atherosclerosis. ROC

**Table 2. Predictors of premature atherosclerosis in univariate and multivariate logistic regression analyses**

| | Models | | | |
|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis | |
| Parameters | OR (95% CI) | *p* | OR (95% CI) | *p* |
| Sex | 1.926 (0.645-5.750) | 0.240 | 1.720 (0.752-3.934) | 0.199 |
| DM | 0.063 (0.008-0.508) | 0.009 | 0.204 (0.046-0.900) | 0.036* |
| HT | 1.257 (0.160-9.903) | 0.828 | - | - |
| Family history | 0.238 (0.083-0.658) | 0.008 | 0.487 (0.224-1.056) | 0.069 |
| Smoking | 0.161 (0.051-0.503) | 0.002 | 0.240 (0.104-0.556) | 0.001* |
| Obesity | 2.984 (0.992-8.975) | 0.052 | 1.611 (0.693-3.742) | 0.268 |
| Mode of delivery | 0.071 (0.010-0.489) | 0.007 | 0.110 (0.025-0.473) | 0.003* |
| Breastfeeding | 1.751 (0.380-8.065) | 0.472 | - | - |
| Antibiotic exposure | 0.295 (0.035-2.456) | 0.259 | - | - |
| High LDL | 1.088 (0.267-4.436) | 0.906 | - | - |
| Low HDL | 0.489 (0.187-1.281) | 0.146 | 0.561 (0.265-1.186) | 0.130 |

p values <0.25 were included in the second model; p<0.05 was accepted as significant in the second model. Obesity: BMI >30 kg/m$^2$, high LDL: LDL >100 mg/dL, low HDL: <40 mg/dL for males and <50 mg/dL for females.
The Hosmer-Lemeshow test was used to check whether the model was appropriate (p=0.490).
*Statistically significant.
CI: confidence interval; DM: diabetes mellitus; HDL: high-density lipoprotein; HT: hypertension; LDL: low-density lipoprotein; OR: odds ratio.




| Variable | AUC | SE | 95% CI |
|---|---|---|---|
| Diabetes mellitus | 0.529 | 0.0225 | 0.456 to 0.601 |
| C-section | 0.607 | 0.0239 | 0.535 to 0.677 |
| Smoking | 0.652 | 0.0337 | 0.581 to 0.719 |

**Figure 2.** Receiver operating characteristic curve analysis of diabetes mellitus, mode of delivery, and smoking.
AUC: area under curve; SE: standart error; CI: confidence interval.

curve analysis determined that C-section was better at predicting premature atherosclerosis than DM, but not smoking, with an area under curve=0.607 and p=0.023 (Figure 2).

The comparison of data for receiving breast milk longer than 1 year showed no significant difference between the 2 groups (p>0.05). There were 2 out of 14 patients with HT, and 5 out of 21 with DM fed with breast milk longer than a year.

The records showed that 8 infants fed with breast milk longer than 1 year received antibiotic treatment (17.7%) compared with 47 (30.2%) who received breast milk for less than a year (p=0.136).

In the subgroup analysis, breast milk intake and duration were longer in patients with atherosclerosis who received medical treatment (6%, never; 7%, 0-6 months; 60%, 6-12 months; and 27%, >12 months in the medically treated group; and 19%, 43%, 26%, and 12%, respectively, in the percutaneously intervened [PCI] or surgery group) (Figure 3). The presence of family history for CAD was statistically different between the medically treated and intervened groups (27% in medical and 69% in coronary intervened group, p=0.002). The C-section delivery rate was higher in the PCI or surgery group, but there was no statistically significant difference (7% in medically treated and 29% in PCI or surgery group, p=0.064) (Table 3). In terms of the Gensini score, breast milk intake duration was lower in patients with higher scores (r=-0.587, p<0.001) (Figure 4).

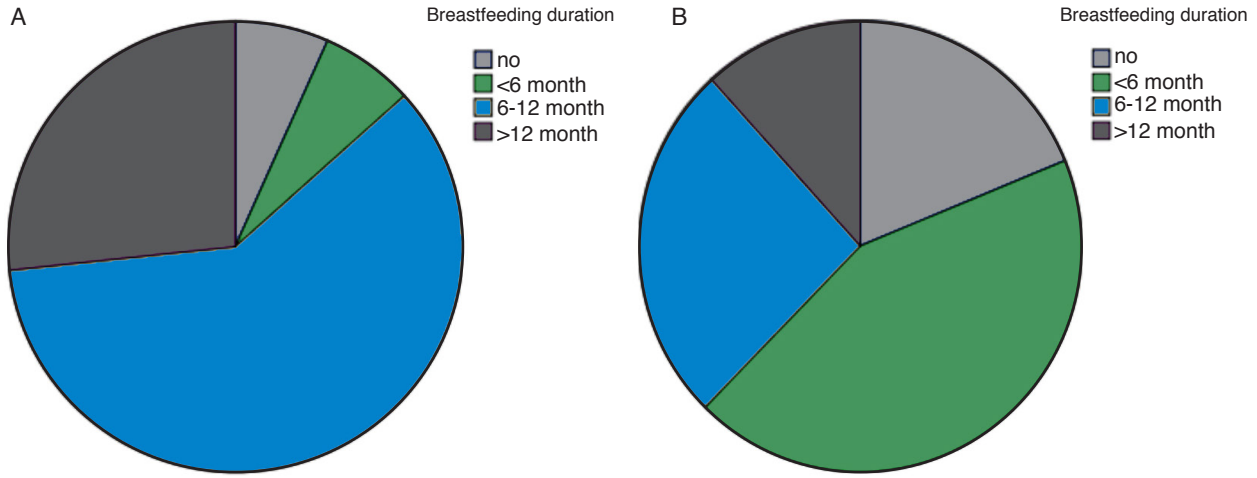



**Figure 3.** Breastfeeding duration in the atherosclerosis group. **(A)** Medical treatment; **(B)** percutaneous intervention or surgery.

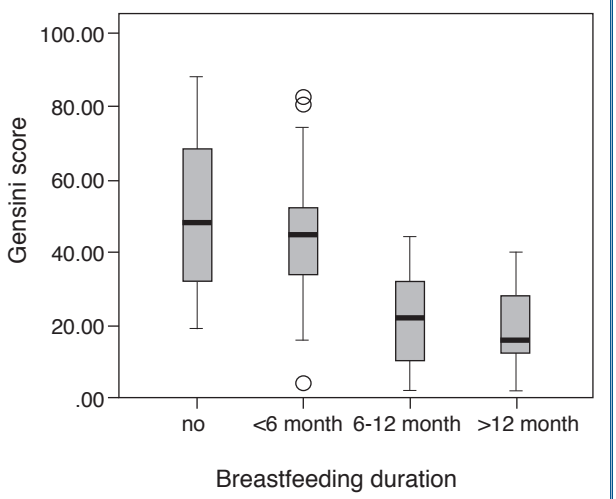



**Figure 4.** Distribution of breastfeeding duration according to Gensini score.

## DISCUSSION

Our study showed that C-section was an independent risk factor for the development of premature atherosclerosis later in life; patients with premature atherosclerosis who had no or short-term breast milk intake during the infantile period experienced more severe disease than those who had long-term breast milk intake; and although not statistically significant, the rate of the antibiotic exposure during the first 2 years of life was higher in the atherosclerosis group than in controls. These results were, to the best of our knowledge, the first in the literature to present an association between C-section, breastfeeding, and atherosclerosis.

There is a mutualistic relationship between microbes and human, which are known as microbiota and have a complex structure. Microbiota, which starts to develop in the early fetal period, has a critical role in the development of gastrointestinal, metabolic, and immune systems. Many factors such as the host diet, lifestyle, host genetics, antibiotic use, and environmental factors are known to affect the composition of the gut microbiota.[11-15] One of these factors is the type of delivery. It has been shown that the newborn is colonized with the vaginal flora in the early period after vaginal delivery, and this occurs later in births via C-section. Early development of gut microbiota has an important role in the development of immune system and immune diseases. The effect of gut microbiota on the development of atherosclerosis as a chronic inflammatory disease has been shown in many studies.[16] Ascher and Reinhardt[17] suggested the composition of the commensal microbiota as an emerging risk factor for CAD, and the use of probiotic strains or fecal microbiota transplantation could become promising therapeutic options to prevent the progression of CAD. The fact that C-section has adverse effects on gut microbiota, the association of the delivery method with chronic diseases might require more in-depth analyses. A meta-analysis by Cardwell et al.[18] has shown that cesarean delivery increases the risk of childhood type 1 DM. However, this hypothesis was not supported in a study by Stene et al.[19] conducted on 1,824 children with type 1 DM.

**Table 3. Characteristics of patients in the atherosclerosis group**

| | Atherosclerosis group (n=100) | | |
|---|---|---|---|
| | Medical treatment (n=15) (mean±SD, n) | PCI or surgery (n=85) (mean±SD, n) | *p* |
| Age, years | 36.2±3.7 | 37.3±2.4 | 0.137 |
| Sex, male, n | 10 | 60 | 0.766 |
| DM, n | 3 | 10 | 0.430 |
| HT, n | 1 | 3 | 0.507 |
| Family history, n | 4 | 59 | 0.002 |
| C/S, n | 1 | 25 | 0.064 |
| Smoking, n | 12 | 63 | 0.628 |
| LDL, mg/dL | 1330.0±21.5 | 147.1±66.5 | 0.489 |
| Gensini score | 8.80±8.25 | 39.06±19.13 | <0.001 |
| Antibiotic exposure, n | | | 0.310 |
| Never | 11 | 56 | |
| Rarely | 1 | 19 | |
| Often | 3 | 10 | |
| Mother's breast milk intake, n | | | 0.006 |
| Never | 1 | 16 | |
| 0-6 months | 1 | 37 | |
| 6-12 months | 9 | 22 | |
| >12 months | 4 | 10 | |

C/S: cesarean section, LDL: low-density lipoprotein cholesterol; SD: standard deviation; DM: diabetes mellitus, HT: hypertension; PCI: percutaneously intervened.

In our study, there was no relationship between the mode of delivery and DM. Unlike our study, Cardwell et al.[18] included studies in their meta-analysis in which only patients with type 1 DM and under the age of 15 years were evaluated. In our study, adult patients were included, and the type of DM was not evaluated. Although type 1 DM, which is associated with the immune system and genetics and seen at a young age, has been found to be associated with C-section; type 2 DM seen in adults may not be associated with C-section.

Martin et al.[20] indicated that breastfeeding was inversely associated with atherosclerosis measured by intima-media thickness and plaque prevalence in a cohort study based on a 65-year follow-up. Rossem et al.,[21] in a follow-up study of 314 children from birth to age 12 years, have demonstrated an association between lower levels of blood pressure and being breastfed. In contrast, Jonge et al.[22] suggested that breast milk intake and duration were not associated with HT. In our study, there was no association of breast milk intake and duration with high blood pressure. However, the patients included in the studies of Rossem et al.[21] and Jonge et al.,[22] who previously examined the relationship between breast milk and HT, were followed up from birth until 12 years and 2 years, respectively; and in our study, the mean age was 37 years. In addition, in both studies, the patients did not have HT, and blood pressure values of all the patients were within normal limits. However, although Rossem et al.[21] found a slight elevation in those who had not been breastfed, Jonge et al.[22] did not. The differences between our study and these studies may have resulted from the fact that the patients were not homogenous, and the definitions were different.

Antibiotics used in the early period of life are known to have harmful effects in addition to benefits of changes in microbiota.[23] Antibiotics used in the first 6 months of life have been shown to be associated with obesity, which is a risk factor for atherosclerosis and DM.[24] It has been shown that obesity developed in mice given sub-therapeutic antibiotics; and the mice in whom the deteriorated microbiota was regulated with probiotics, were protected against obesity.[25] Rautava[26] suggested that exposure to antibiotics during the prenatal and infantile period increased the risk of chronic diseases. There was no association between antibiotic use in the infantile period and the adulthood atherosclerosis in our study. However, the results showing that patients fed breast milk longer than a year had less need for antibiotic treatment, might indicate a superiority of being breastfed over a longer duration and attract attention for further investigations regarding the link between breast milk, antibiotic use in the infantile period, and adulthood atherosclerosis. Like many other morbidities, comorbidities generally develop into more severe medical conditions for patients.

Neu and Rushing[27] have claimed that the babies born via C-section and those who received antibiotic treatment in infancy might have disrupted the core microbiome and experience diseases such as obesity, diabetes, allergy, celiac, and asthma. Our results showed that, according to multivariate logistic regression analysis, a trend toward significance for C-section to predict the development of CAD was

observed; and the duration of breastfeeding was determined to be an independent predictor of the severity of the disease.

In our study, we found that the Gensini score was higher in patients who were breastfed for a shorter time. Because there are no studies evaluating breast milk intake and duration, type of delivery, or antibiotic exposure with this scoring system in the literature, it is appropriate to evaluate this issue with studies involving a large patient population.

## Limitations

The study was confined to a single-center, and the sample size was limited. Another significant limitation was the accuracy of the anamnesis of the cases; although the families had confirmed the history taken from the patients, the specifics of breastfeeding and antibiotic use may have been false recalled. Conventional angiography has limitations in evaluating early coronary atherosclerosis; therefore, the lack of confirmation of the coronary disease with intravascular ultrasound or optical coherence tomography could be considered as a limitation. Finally, because patients with premature atherosclerosis aged below 40 years were included in the study, the results cannot be generalized to all patients with atherosclerosis.

## Conclusion

Our results showed that C-section as the mode of delivery is an independent risk factor for the development of premature atherosclerosis later in life, and patients with premature atherosclerosis who had not taken breast milk or were breastfed for less than 3 months had more severe coronary disease. Therefore, the mode of delivery and the history of breast milk intake require further investigations and should be considered as prospective additional risk factors for premature atherosclerosis and become essential parts of the cardiovascular risk assessment and clinical evaluation.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Clinical Research Ethics Committee of Adana City Training and Research Hospital (Approval Date: January 8, 2020; Approval Number: 664).

**Peer-review:** Externally peer-reviewed.

**Authorship contributions:** Concept - Ö.Ö.A.; Design - Ö.Ö.A.; Supervision - Ö.Ö.A., S.K.; Materials - Ö.Ö.A.; Data - Ö.Ö.A.; Analysis - Ö.Ö.A.; Literature search - Ö.Ö.A., M.K., A.Y., M.K., S.K.; Writing - Ö.Ö.A., M.K., A.Y., M.K., S.K.; Critical revision - Ö.Ö.A., S.K.

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