Acute atherosis of decidua basalis: characterization of spiral arteries, endothelial status and activation

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Keywords

Decidua basalis, acute atherosis, atherosclerosis, endothelial activation, endothelial damage, CD31 intensity
Abstract

Introduction

Acute atherosis (AA) is a lesion affecting uteroplacental spiral arteries during pregnancy, most frequently in preeclampsia but occasionally in normal pregnancy. It is commonly observed in untransformed spiral arteries (intact smooth muscle cell layer, lacking intramural trophoblasts). The mechanism causing lesion development is unknown. AA shares some morphological similarities with atherosclerosis, in which endothelial activation occurs early. Here we histologically characterize decidua basalis spiral arteries with and without AA, focusing on endothelial status and activation.

Methods

Formalin-fixed and paraffin-embedded decidua basalis tissue sections from 32 patients (16 normotensive, 5 with AA, 16 preeclampsia, 7 with AA) were stained with H+E, PAS, MSB (Martius Scarlet Blue), desmin, CK7, CD68, CD31, vWF and ICAM-1. We logged remodeling status, presence of AA, endothelial morphology, endothelial CD31 intensity and activation (ICAM-1-positive cells).

Results

We observed fully or partially transformed spiral arteries in most decidua basalis samples, and no untransformed arteries. AA arteries were also observed, characterized by intramural CD68-positive vacuolated cells and fibrinoid necrosis. They lacked a smooth muscle cell layer and intramural trophoblasts. The fibrinoid necrosis in AA lesions stained red with MSB. AA arteries were associated with lower CD31 staining intensity of endothelial cells. More arteries had an abnormal or destroyed endothelium relative to arteries without AA. Endothelial activation was not observed in the majority of AA arteries.
Discussion

Our results indicate an altered endothelial phenotype as important in the development of AA, supporting previous observations. The histology of AA differs from that of atherosclerosis.

Introduction

Acute atherosis (AA) affects the uteroplacental spiral arteries, which perfuse the placental intervillous space during pregnancy. AA occurs in 10-40% of preeclamptic pregnancies [1, 2], but also in a lower proportion of normotensive pregnancies [2, 3]. It has been described in spiral arteries in the myometrium, decidua basalis, placental basal plate, and decidua parietalis [2, 4-6]. The lesion is commonly associated with incomplete spiral artery remodeling (intact smooth muscle cell layer, lacking intramural trophoblasts) [7, 8], also described as incomplete physiological transformation.

AA is characterized by intramural CD68-positive vacuolated cells (“foam cells”), thus appearing morphologically similar to early atherosclerosis lesions [9, 10]. Further, it is associated with fibrinoid necrosis and occasionally a mononuclear perivascular infiltrate [1, 4].

The mechanisms of AA are not understood. It is not clear whether the lesions in the myometrium, decidua basalis and parietalis represent the same pathological processes, even though they share some similar features. Based on the similarity to atherosclerosis, which is established as an inflammatory vascular lesion, we have proposed that vascular inflammation may also be associated with the development of AA [9]. Initial stages of atherosclerosis include endothelial activation, as demonstrated by an increased expression of adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) [11]. Expression of these
molecules promotes attachment and recruitment of circulating monocytes into the vessel wall [12].

This study gives a detailed and systematic histological characterization of decidua basalis spiral arteries with and without AA, to determine whether endothelial status and activation is consistent with our hypothesis.

Methods

Patient recruitment and tissue collection

Pregnant women were recruited as described previously [1], and all gave informed written consent. Preeclampsia was defined as new onset of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (≥1+ on dipstick or ≥30 total protein/creatinine ratio) at ≥20 weeks of gestation [13]. None of the women had regular uterine contractions or signs of infection, and membranes were intact at time of delivery. None had known chronic diseases. Decidua basalis tissue was obtained by vacuum suction of the placental bed following elective caesarian section, as described previously [14]. The collected tissue was fixed in buffered formalin and embedded in paraffin (FFPE).

Decidua basalis tissue evaluation

For all patients, FFPE tissue sections (3 µm) of the decidua basalis stained for desmin, CK7, and CD68, counterstained with Periodic Acid-Schiff (PAS), were available [1]. Decidua basalis origin was confirmed by the presence of CK7-positive interstitial trophoblasts and decidual cells. Spiral arteries with complete physiological transformation (fully remodeled) were identified by the presence of CK7-positive trophoblasts in the vessel wall, of intramural fibrinoid (bright purple upon PAS staining) and of complete absence of mural smooth muscle cells (no desmin stain) [15]. Partially transformed spiral arteries (partially remodeled) had both intramural fibrinoid and trophoblasts, and scattered areas with remaining mural smooth
muscle cells (desmin-positive). Acute atherosclerosis (AA) arteries were defined as arteries with ≥2 intramural adjacent vacuolated CD68-positive cells in the vessel wall [1]. Vessels with an intact desmin-positive smooth cell muscle layer and absence of intramural trophoblasts were defined as untransformed spiral arteries (unremodeled) or basal arteries (typically <100µm in diameter) [15]. Only arteries with a diameter of at least 140 µm were included in order to exclude basal arteries from the analysis. Arteries with AA lesions were categorized as AA arteries. Arteries lacking AA lesions were categorized as nonAA arteries. Decidua basalis samples lacking AA arteries were categorized as nonAA samples. Decidua basalis samples harboring ≥1 artery with AA were categorized as AA samples. AA is a focal lesion, seldom affecting all arteries in a sample, so nonAA arteries in samples also harboring AA arteries were defined as nonAA arteries in AA sample. In total 32 patients were included in the study, 16 normotensive controls (NC) (5 with AA, which is a purposeful overrepresentation of the 10% AA rate we have previously found in controls [1]) and 16 with preeclampsia (PE) (7 with AA). The clinical characteristics of the patient groups are presented in Supplementary table 1.

Immunohistochemical and histological staining

Additional 3 µm serial slides were stained with CD31 (PECAM-1) (Dako, mouse, clone JC70A, 1:400), von Willebrand Factor (vWF, Dako, rabbit polyclonal 1:120000) counterstained with PAS, and intercellular adhesion molecule-1 (ICAM-1, mouse, clone 23G12, Thermo Fisher Scientific, 1:10+amplification) using a Ventana XT autostainer (Roche) following the manufacturer’s protocol. All reagents were obtained from Roche. Pictures were obtained using an Axio Scan slide scanner and Zen Blue software (Zeiss). Specificity of antibody staining was verified using appropriate positive and negative controls. An example of positive and negative controls for ICAM-1 is shown in Supplementary Figure
1. Serial slides were stained with Martius Scarlet Blue (MSB) [16] and Hematoxylin+Eosin (H+E) using standard histological techniques.

**Evaluation of endothelial status and activation**

Endothelial cells in spiral arteries were identified by positive CD31 and vWF staining. The histological appearance could be divided into three main categories; “Normal”; flattened CD31-positive endothelial cells lining the vessel wall, “Abnormal”; CD31-positive endothelial cells, but more irregular in shape than normal flat endothelium, and “Destroyed”; the endothelial cell layer is disrupted and detached from the vessel wall or partly lacking. Arteries with mixed endothelial cell appearance (abnormal+normal) were classified as abnormal if >25% of the endothelium had this phenotype.

For each vessel, we also evaluated intensity of CD31 staining across the endothelium. The Image Analysis module of the Zen Blue software (Zeiss) was used to identify and quantify the areas of high and low CD31 intensity for the endothelium lining each vessel. Thresholds for high and low intensity of stain were set manually in Image Analysis in Zen Blue, after ensuring similar intensity level between each scanned slide. NonAA vessels were used to define high intensity level. An example of the image analysis is shown in Supplementary Figure 2.

Endothelial activation was defined as ≥3 consecutive ICAM-1-positive endothelial cells, as used previously in a study of endothelial activation in atherosclerosis [17]. Samples were evaluated without knowledge of the patients’ clinical data.

**Statistics**

The data were analyzed using SPSS Statistics 25.0 (IBM). For continuous variables a non-parametric Mann-Whitney U test was used. Fisher’s exact test was used for categorical variables, expected cell count <5. A p-value <0.05 was regarded as significant.
Results

Decidual basalis spiral artery identification and characterization

We identified a total of 124 spiral arteries across all evaluated samples. The data were primarily analyzed by artery not by case, as the focus of this study was the histology of the AA lesion. The arteries were categorized according to presence of AA (AA artery) or not (nonAA artery) as described in the methods section (Table 1).

The median number of spiral arteries observed per decidua basalis tissue section was higher in normotensive controls (NC) than preeclampsia (PE) (Table 1), however the difference was not significant. There were no significant differences in the median number of spiral arteries observed in nonAA samples vs AA samples for either NC or PE groups. Spiral arteries with complete physiological transformation (Figure 1a) were observed both in NC and PE samples (Table 1), with no significant difference in the fraction of arteries with complete physiological transformation per sample between PE and NC. In AA samples, the fraction of AA arteries per total arteries observed was similar between NC and PE (Table 1).

In AA samples, we also frequently observed completely transformed arteries (60% of NC and 71% of PE cases). The fractions of completely transformed arteries per total arteries in AA samples were similar between NC and PE, and it was significantly lower than for nonAA samples for both NC and PE. Partially transformed spiral arteries (Figure 1b) were less frequently observed, with no difference in frequency between study groups (Table 1). Arteries with an intact desmin-positive smooth muscle cell layer were rarely observed, and excluded as basal arteries because of their size (<140 µm). None of these had AA lesions. We did not observe any non-remodeled arteries with a diameter >140 µm.

AA arteries were always associated with fibrinoid necrosis; visualized as areas of grey-pink PAS stain in the vessel wall, which were also bright red with MSB staining. These arteries lacked the bright purple fibrinoid PAS-stain of completely transformed spiral arteries (Figure...
In contrast, red MSB stain was not observed in completely or partially transformed arteries (nonAA arteries), except for a few cases where some traces were observed. For most AA arteries we observed no intramural CK7-positive trophoblasts, and only remnants or complete absence of desmin-positive smooth muscle cells (Figure 1c), as reported previously [1]. However, for three AA arteries across three different patients (2 PE, 1 NC) we observed foam cells and fibrinoid necrosis in combination with trophoblasts and normal PAS-positive purple fibrinoid in the same artery (Figure 1d).

For spiral arteries with complete or partial physiological transformation, individual study variables were distributed similarly regardless of remodeling status (Supplementary table 2), and these arteries were merged into one nonAA artery category. The subdivision of the nonAA artery category into nonAA artery in nonAA sample and nonAA artery in AA sample (was done in order to detect potential differences in the same vessel category (nonAA artery) between samples with and without AA arteries. Arteries from NC and PE were merged for plotting and statistical analysis, as they displayed a similar distribution across the individual study variables (Supplementary table 2 and 3).

AA is associated with an altered endothelial phenotype

Spiral artery endothelium was identified by positive CD31 and vWF staining. The histological appearance was classified as normal (Figure 2a), abnormal, but not destroyed (Figure 2b, c, d), or destroyed (Figure 2e), as defined in the methods section. The distribution of the three spiral artery categories across the endothelial subtypes is shown in Figure 3a.

NonAA arteries, both in nonAA samples and AA samples, were equally distributed between a normal and abnormal endothelial cell pattern and few had a destroyed phenotype (Figure 3a and Supplementary table 2). In contrast, significantly less of the arteries with AA (AA arteries in AA sample) had a normal endothelial phenotype, and most of the AA arteries (50%) were
categorized as abnormal. For the remaining 30% of the AA arteries a destroyed phenotype was observed, which was significantly higher relative to both nonAA artery categories (Figure 3a).

AA is associated with weaker CD31 staining of abnormal endothelium

There were differences in CD31 staining intensity between arteries (Figure 2), and CD31 staining intensity of areas of intact endothelium was categorized as either high or low (Supplementary Figure 2), as outlined in the methods section. The percentage of vessel endothelium with a low CD31 intensity relative to total area of endothelium for each vessel across different spiral artery categories and endothelial subtypes is shown in Figure 3b.

Quantification of CD31 intensity for the destroyed phenotype was not performed, as the endothelial cells were destroyed and disintegrated. For the normal endothelial phenotype, no significant differences in CD31 intensity were observed between any of the spiral artery categories (Figure 3b). For the abnormal endothelial phenotype, AA arteries had a higher percentage of areas with low CD31 intensity relative to total endothelium per vessel. This was significantly more than for both nonAA artery in nonAA sample and nonAA artery in AA sample categories (Figure 3b), however the endothelial lining was still intact and present for all vessel categories.

The vWF staining of endothelial cells appeared in general weaker than for CD31, possibly because of the PAS counterstain (Supplementary Figure 3). We did not observe a clear difference in staining intensity for vWF between nonAA and AA arteries, unlike our observations regarding CD31.

Endothelial activation is not detectable in a majority of AA vessels

Almost all decidua basalis spiral arteries had some scattered ICAM-1-positive endothelial cells, so endothelial activation was defined as presence of ≥3 adjacent ICAM-1-positive
endothelial cells (Figure 2d), as used previously in a study on atherosclerosis [17]. None of the vessels with destroyed endothelium could be evaluated for ICAM-1, as the endothelium was destroyed and disintegrated. Within the normal endothelial phenotype there was a tendency towards higher endothelial activation in the AA arteries (constituting 16.7% of all AA arteries), however the numbers were very low (only 4 observations, of which 3 were positive), and the difference not statistically significant.

There were no significant differences in endothelial activation between the spiral artery categories within the abnormal endothelial phenotype (Figure 3c), only 4/12 (33%) of the AA vessels with abnormal phenotype had endothelial activation.

**Discussion**

The characteristics of AA at different sites in the uteroplacental unit have been investigated previously [18-21]. However, only a few studies [19, 20] have histologically explored the morphology of AA by thoroughly characterizing spiral arteries in the decidua basalis. Our study of samples collected by vacuum suction after removal of the placenta during elective cesarean section is unique.

Our first observation is that AA arteries are associated with an altered endothelial phenotype. A majority of the AA arteries (50%) were associated with abnormal endothelium with weaker CD31 staining relative to arteries without AA, or a damaged endothelium (30% of AA arteries). Endothelial damage associated with AA has previously been described both in the myometrium, decidua basalis [21, 22], as well as in the decidua parietalis, as observed by Hecht et al [20]. Hecht et al also used endothelial markers; however they did not report alterations in staining intensity [20]. Positive red staining with MSB in the AA artery wall, as observed by us and others [7, 19], indicates deposition of fibrin (or fibrin-like material) most
likely caused by a leakage of factors from the maternal circulation into the vessel wall possibly because of an altered endothelial phenotype [22].

The causes of reduced CD31 staining in AA arteries require further investigation. The reduced staining could indicate that the CD31-positive cells are in a state of disintegration, which could also affect ICAM-1 staining. However, in some cases we observed both weak CD31 stain as well as positive ICAM-1 stain. Weaker CD31 staining in AA arterial endothelium could indicate an altered endothelium as a first response to damaging or inflammatory stimuli. Cellular stress responses could affect global protein translation [23] causing lower CD31 staining. This would not affect ICAM-1 translation as this protein is selectively translated during stress conditions via the ATF4 transcription factor. As we do not observe clear reduction in vWF staining, it could indicate that the reduction in staining is not caused by general endothelial cell degeneration.

We were in general not able to find an association between endothelial activation (ICAM-1-positive stain) and presence of AA in decidua basalis arteries. For arteries with abnormal endothelium (constituting 50% of the AA arteries) endothelial activation was only observed in 25% of the arteries. In 30% of the AA arteries (destroyed phenotype) we could not assess ICAM-1 staining because the endothelial cell lining was completely disintegrated or partly lacking). For AA arteries with a normal endothelial phenotype (constituting only 16.7% of AA arteries), a trend towards higher ICAM-1 staining was observed; 3/4 (75%) were ICAM-1-positive, however because of the low numbers of observations the trend was not significant.

Even though we did observe endothelial activation in some AA vessels, especially for the normal phenotype, it rarely affected the whole circumference of the vessel, and it was not clearly associated with the location of the foam cells embedded in the vessel wall. The overall histological appearance of AA arteries differs from atherosclerotic lesions where an intact endothelial cell layer is observed with clear ICAM-1-positive staining, and without deposition
of fibrin, even in the advanced stages of the lesion [11, 17, 24, 25]. In summary, our data suggest that the decidual basalis AA lesion is associated with an altered endothelium; however it differs histologically from the observations found in atherosclerosis. This is in line with the conclusions of Katabuchi et al, in their characterization of the AA lesion [19]. Even though AA in our material is histologically dissimilar from atherosclerosis, we cannot exclude from our findings that ICAM-1-positive staining is present at an earlier stage of the AA lesion formation.

A recent study of endothelial activation in spiral arteries from the placental basal plate [5] showed that for arteries in preeclamptic samples, with complete failure of remodeling, both the arterial endothelium and the surrounding trophoblasts labelled for ICAM-1. AA was only observed in placentas harboring ICAM-1-positive vessels; however the investigators did not report whether ICAM-1-positive endothelial cells and AA localized in the same artery, nor the endothelial status. In contrast, we observed no ICAM-1-positive trophoblasts in the decidua basalis, and very few arteries with complete failure of physiological transformation (intact smooth muscle cell layer and absence of intramural trophoblasts), none with a diameter >140 μm. We did not assess ICAM-1 status for excluded vessels. A further difference from this and other reports [15, 26], is that we find that spiral arteries in decidual samples from PE patients in our cohort were predominantly fully remodeled. It is possible that the absence of unremodeled arteries in PE samples in our study could be because the PE cases included are less severe, as we have a mix of early and late PE with and without fetal growth restriction, with no preexisting hypertension. The observation of completely unremodeled spiral arteries in the placental basal plate at term by Labarrere et al [5] would point towards a more severe preeclampsia phenotype. However, the clinical data for the PE group and information of co-morbidities are not included in the Labarrere paper [5]. A previous study by Tziotis et al, did not find any significant difference in level of ICAM-1 and other markers of endothelial
activation between preeclampsia and normotensive pregnancies in samples from the placental bed [27].

Our finding of lack of a smooth muscle cell layer in AA vessels is in line with a previous report by Katabuchi et al [19]. The processes causing the lack of smooth muscle cells in these vessels cannot be established from our work, but smooth muscle cells necrosis or apoptosis is likely a major cause, as suggested by Katabuchi et al. We cannot exclude that the smooth muscle cell layer was present at the time of lesion initiation. In line with existing literature [26], we observe a lack of intramural trophoblasts in arteries with AA lesions [1]. The lack of intramural trophoblasts and trophoblast-derived fibrinoid indicate that the vessels were not remodeled prior to AA formation.

It is noteworthy that we observe AA arteries (lacking intramural trophoblasts) and completely transformed arteries (with intramural trophoblasts) in close proximity to one another. In completely transformed arteries, bright purple PAS-positive fibrinoid is observed in the vessel wall, which is most likely trophoblast-derived [8, 22]. In contrast, AA arteries lack the purple PAS-positive fibrinoid, instead fibrinoid necrosis (grey-pink PAS-stain) is observed, which also stains bright red with MSB staining. In a few cases, we observed a mix of both features, with part of the vessel displaying full physiological transformation with intramural trophoblasts, and the other part having foam cells and red MSB stain in-between the endothelium and a normal outer vessel wall. This suggests that local factors cause AA since neighboring arteries display a normal physiological remodeling status, and in some cases only part of the vessel is affected.

Our data from vacuum suction samples of decidua basalis complements earlier studies of placenta bed biopsies, placenta basal plates and decidua parietalis tissue. The major strength of this study is our vacuum suction method, which yields a higher number of spiral artery
sections compared to placenta bed biopsies and placenta surface biopsies [3]. Our systematic immunohistochemical and histological staining enables a clear visualization of the histological and cell specific composition of AA lesions and neighboring arteries. Of necessity, our study is of a relatively low number of patients, owing to the extensive staining and evaluation protocol.

In summary, our observations indicate that AA is a focal lesion, associated with an altered endothelial phenotype and lack of intramural trophoblasts in the affected arteries only. We are not able to establish whether an altered endothelial phenotype precedes and potentially inhibits endovascular trophoblast invasion, or if lack of trophoblast invasion would lead to endothelial alteration and AA formation. As AA is also observed in spiral arteries of decidua parietalis, where no trophoblast invasion takes place, it is tempting to speculate that the endothelial alterations may be the initiating events. We were not able to detect ICAM-1 positive staining of the endothelium in a majority of AA arteries, dissimilar from what is observed for atherosclerosis.

Conflict of interest statement

The authors declare that they do not have any conflict of interest

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References


Figure legends:

Figure 1. Staining of serial FFPE sections of decidua basalis tissue to identify spiral arteries. Slides are stained with (from left to right) Hematoxylin+Eosin (H+E), desmin+PAS, CK7+PAS, CD68+PAS and Martius Scarlet Blue (MSB). Representative images of a) a spiral artery from a normotensive control with complete physiological transformation, characterized by presence of CK7-positive trophoblasts and intramural fibrinoid (bright purple upon PAS staining, white arrowhead) in the vessel wall, and complete absence of intramural smooth muscle cells (no desmin stain). b) Spiral artery from a PE patient with partial physiological transformation (both intramural fibrinoid and trophoblasts (CK7-positive) as well as areas with traces of mural smooth muscle cells (desmin-positive)). c) Spiral artery with acute atherosis from same sample as in b), lacking bright purple fibrinoid and CK7-positive trophoblasts in the vessel wall. Traces of intramural smooth muscle cells (desmin positive) are seen. Fibrinoid necrosis is visible as a grey-pink material in the vessel wall (asterisk), which stains red upon MSB staining (asterisk). Erythrocytes in the lumen of the AA artery stains red-brown upon MSB staining. Intramural CD68-positive foam cells are present (black arrowhead). d) Spiral artery from a PE patient with almost complete physiological transformation (lack of desmin-positive smooth muscle cells, presence of CK7-positive trophoblasts), yet acute atherosis lesion present (asterisk; fibrinoid necrosis, black arrowhead; foam cells, white arrowhead; purple physiological fibrinoid). Inset; higher power inset of foam cells.
**Figure 2. Staining of serial FFPE sections of decidua basalis tissue to evaluate endothelial status and activation.** Slides are stained with (from left to right) Hematoxylin+Eosin (H+E), CD31, and ICAM-1. a) and b) are vessels from the same PE patient sample. The full staining for vessel d) is shown in Figure 1c. a) NonAA-artery: normal endothelium with high intensity CD31 stain. Single ICAM-1-positive cells, but no endothelial activation. b) (PE) NonAA-artery: abnormal endothelium (arrowhead) in combination with normal endothelium. c) Example of AA-artery from a PE patient without endothelial activation: intact, but abnormal endothelium with low intensity CD31 stain. No ICAM-1
positive stain. d) Example of AA-artery with endothelial activation: intact, but abnormal endothelium with low intensity CD31 stain. Endothelial activation (≥3 consecutive ICAM-1-positive cells). e) (PE) AA-artery: destroyed endothelium, no endothelial activation.

Figure 3. Spiral artery and endothelial cell characterization across vessel categories. a) Distribution of spiral artery categories across the endothelial cell phenotypes (Normal, abnormal and destroyed). Bars show the percentage of arteries in each artery category associated with each endothelial subtype. NonAA-arteries are shown as light grey bars, nonAA-arteries in AA samples are shown as darker grey bars and AA-arteries (in AA samples) are shown as black bars. b) Percentage of area of low CD31 intensity relative to total area of endothelium per vessel for each artery subtype across the normal and abnormal endothelial cell categories. c) Percentage of vessels with positive ICAM-1 stain (≥3 consecutive cells) for each artery subtype across endothelial cell categories.
Table 1: Observed distribution of spiral arteries and categories across the study groups. The total number of spiral arteries observed within each study group, as well as the number of nonAA and AA arteries. The median number (and range) of spiral artery sections observed per evaluated tissue section for each study group. NC=Normotensive controls. PE= preeclampsia. nonAA sample; sample lacking artery with acute atherosis (AA), AA sample; sample containing ≥1 artery with acute atherosis. The distribution (in percentage) of completely transformed, partially transformed and AA arteries of the total number of spiral arteries observed per tissue section is presented as median (and range) across the samples in each category. NonAA arteries= arteries with complete or partial physiological transformation without AA.

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Statistical differences were calculated for the total group of normotensive controls (NC) against preeclampsia (PE), and within the NC and PE group for samples without acute atherosis (nonAA sample) against women with acute atherosis (AA sample). We compared medians of continuous variables by non-parametric Mann-Whitney U test. Differences were considered to be statistically significant when p<0.05. <sup>a</sup> Significantly different when comparing nonAA sample vs AA sample for NC group. <sup>b</sup> Significantly different when comparing nonAA sample vs AA sample for PE group.