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Premature Atherosclerosis in Premenopausal Women: Does Cytokine Balance Play a Role?

Jo-Ann Eastwood, PhD,

University of California Los Angeles

Doris A Taylor, PhD,

Texas Heart Institute, Houston

B. Delia Johnson, PhD,

University of Pittsburgh

Micheline Resende, PhD.,

Texas Heart Institute

Barry L. Sharaf, MD.,

Rhode Island Hospital

Bina Ahmed, MD,

Dartmouth-Hitchcock Medical Center

Margo Minissian, PhD,

Barbra Streisand Women's Heart Center, Cedars-Sinai Heart Institute

Chrisandra Shufelt, MD, MS, and

Barbra Streisand Women's Heart Center, Cedars-Sinai Heart Institute

C. Noel Bairey Merz, MD

Barbra Streisand Women's Heart Center, Cedars-Sinai Heart Institute

Abstract

Contributory risk factors to premature coronary artery disease (CAD) in premenopausal women are poorly understood and data on this subset of women is lacking. There is growing evidence that the process of inflammation is a part of the atherosclerotic process. Mechanistic insights from animal work suggest that the profile of circulating cytokines reflects both endothelial integrity and the presence of immune and progenitor cells. Significant differences in pro- and anti-inflammatory cytokine concentrations between patients with and without CAD exist. Young women with obstructive CAD may experience differences in pro-inflammatory cytokines and the recruitment of reparative cells that secrete T-Helper (TH2) cytokines compared to women without CAD. Thus,

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Address for correspondence: Jo-Ann Eastwood PhD, University of California, Los Angeles, Factor Building 4-266, 700 Tiverton Ave. Box 956918, Los Angeles, California 90095, Fax: 310-794-7482, jeastwood@sonnet.ucla.edu.

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cytokine balance may play a role in obstructive CAD in young women. In this pilot study we set out to identify an array of circulating inflammatory marker profiles which could be useful for the development of risk assessment and preventive strategies. We tested the hypothesis that an increase in serologic TH1 cytokines relative to TH2/hematopoietic regulatory (HR) cytokines is related to premature coronary atherosclerosis in premenopausal women.

INTRODUCTION

Contributory risk factors to premature coronary atherosclerosis in premenopausal women are poorly understood. Over the last two decades, inflammation has been confirmed as a key mediator of atherogenesis.(1–3) In addition, an evolving hypothesis of atherosclerosis is a disease of failed endogenous vascular repair.(4) Serologic T helper 1 (TH1-type pro-inflammatory) and hematopoietic/regulatory (HR) cytokines and their counteraction by TH2-type (anti-inflammatory) appear to relate with the degree of atherosclerosis in animal models.(5) Consistent with this hypothesis, cytokine balance is related to endothelial integrity and is likely mediated by circulating cells.(4) Mechanistic insights from animal work suggest that the profile of circulating cytokines reflects both endothelial integrity and the presence of immune and progenitor cells.(4) Significant differences in pro- and anti-inflammatory cytokine concentrations between human subjects with and without obstructive coronary artery disease (CAD) have been reported.(6)

Vascular injury generates an inflammatory response that triggers the recruitment of “detrimental” cells, including dendritic cells, T lymphocytes and type 1 macrophages, leading to tissue damage. (7) In response to this inflammatory state, and the secretion of pro-inflammatory chemo/cytokines, hematopoietic stem cells (HSCs), type 2 macrophages, and other cells that mediate repair, and renew the endothelium are mobilized ((4). Although these cells secrete anti-inflammatory chemokines such as interleukin 3 (IL-3) and IL-8, if the TH1 secreting cells predominate, ensuing tissue damage leads to lesion progression.(4) However, as reparative cells are recruited they secrete anti-inflammatory chemo/cytokines that counteract the pro-inflammatory status and can augment repair in activated segments of the endothelium.(8) (4)

Atherosclerosis is apparent at a relatively later age in women compared to similarly aged men.(9) In women, obstructive CAD is more frequently associated with autoimmune diseases, which suggests that the immune mechanisms for the development of CAD are different in men and women.(10) Furthermore women have more non-obstructive coronary atherosclerosis than men, which may suggest that key differences in inflammatory processes between obstructive and non-obstructive CAD may exist. (11) This has not been investigated to date.

The hypothesis

An increase in TH1 cytokines relative to TH2/HR cytokines is related to premature coronary atherosclerosis in premenopausal women. The objective of this pilot study was to compare the levels of TH1, TH2 and HR cytokines in premenopausal women with and without obstructive CAD using core laboratory determinations.

METHODS

The study was approved by the Cedars-Sinai Medical Center and the University of California Los Angeles Institutional Review Boards. All patients gave informed consent. Premenopausal women with chest pain undergoing coronary angiography for suspected cardiac ischemia were included. Pre-menopause was determined by published criteria established by the Women's Ischemia Syndrome Evaluation (WISE) based on hormone levels and cycling history (12). Exclusion criteria were: 1) pregnancy, 2) currently using hormone replacement therapy, oral contraceptives, estrogen, or phytoestrogen treatments and 3) the presence of any co-morbidity (i.e., cardiomyopathy, valvular disease).

Procedures

A WISE core laboratory (Rhode Island Hospital, Providence, RI, USA), blinded to clinical data, analyzed all angiograms to characterize the presence and extent of CAD. Obstructive CAD was defined as $\geq 50\%$ stenosis in any epicardial coronary artery, non-obstructive CAD was defined as $\geq 20\%$ but $<50\%$, and no CAD was $<20\%$ (13).

Menopausal status was confirmed by baseline and second visit hormone levels that were timed to coincide with the luteal phase of the menstrual cycle. Blood was collected at visit two to evaluate plasma levels of 22 chemokines/cytokines and endothelial function markers. Chemo/cytokines were measured at a core lab at the University of Minnesota as previously reported by using a cytokine multiplex platform (Human Fluorokine MAP Base Kit; R&D Systems). (4) The detectable chemo/cytokines in our analysis were grouped as TH1 or TH2 as follows: TH1 (pro-inflammatory) interleukin-1 receptor antagonist (IL-1ra), Regulated on activation, normal T expressed and secreted (RANTES), macrophage inducible protein 1 (MIP-1b) and tumor necrosis factor alpha (TNF α); TH2 monocyte chemoattractant protein 1 (MCP-1). The regulatory cytokines and mobilizing chemo/cytokines detected were interleukin 6.

(IL-6), Granulocyte colony-stimulating factor (G-CSF), Vascular endothelial growth factor (VEGF), thrombopoietin (TPO) and epithelial-derived neutrophil-activating peptide-78 (ENA-78). IL-6 was included in the TH2 category although it is sometimes considered to be in the TH1 (anti-inflammatory) group. RANTES was included because it is often associated with a TH1 environment.

Group comparisons of demographic and clinical variables between women with and without CAD were made using nonparametric Kruskal-Wallis for continuous measures and Fischer exact tests for frequencies. Median z-scores for each cytokine were used to create comparative cytokine profiles of women with and without obstructive CAD. Z-Scores were used because they are unit-non-specific and allowed for a comparison across cytokines. Nonparametric Kruskal-Wallis was used because of the small N and the high variability in the cytokines (Table 2). For a visual comparison, a Rose-of-Wind diagram was created depicting the differences in cytokine concentrations. All analyses were performed with SAS 9.3 (SAS Institute, Cary, North Carolina. The significance level was set at .05.

RESULTS

Twenty-four women (mean age 44 ± 5 years) met the study inclusion criteria and comprised the final sample. Patient demographics, clinical characteristics and traditional risk factors of women with versus without obstructive CAD are displayed in Table 1. Of note, some of the differences between the two groups are quite large, although they were not statistically significant.

Among the six TH1/TH2 ratios available for comparison, three ratios (ENA/MCP, IL6/MCP, and RANTES/MCP) were lower in women with obstructive CAD compared to women without obstructive CAD (Table 2), indicating higher ratios of anti-inflammatory/reparative cytokines. Further, an examination of TH1 (pro-inflammatory) and TH2/HR (anti-inflammatory/reparative) patterns in women with and without obstructive CAD demonstrated that lower TH1 cytokines predominated in women with obstructive CAD, while both groups had similar levels of TH2/HR cytokines (Figure 1, $p < 0.05$).

DISCUSSION of the HYPOTHESIS

This pilot study suggests that women with obstructive CAD have lower pro-inflammatory and anti-inflammatory (TH1/TH2) ratios compared to women without CAD – the opposite of our hypothesis. These data suggest the alternative hypotheses that young women without obstructive CAD have better “remodeling” responses or that they have subclinical non-obstructive atherosclerosis and coronary microvascular dysfunction (13) reflecting latent inflammation, which may lead to future obstructive CAD. (14)

These results may extend recent work of cytokine patterns differing by age, sex and hormone levels which contribute to cardiac remodeling, with TH2 cytokines believed to play a predominant reparative role. (11) Furthermore, prior data showed higher ENA-78/MCP ratios in young women after an ischemic event. (14) There is extensive knowledge of inflammatory phenomenon and atherosclerosis. (15, 16) Our current findings differ from previous longitudinal studies, in which investigators reported higher TH1 levels in CAD patients, particularly those evaluated early after an acute event. (17) This may be attributable to several factors. First, women with obstructive CAD in our sample had not experienced recent acute events. As a result, they may have been experiencing a protective immune response in which a significant rise in TH2/HR cytokines counters levels of TH1 cytokines. Second, this finding may also be related to statin administration. Evidence exists as to the reversion of pro-inflammatory oxidative functional responses after treatment with statins in patients with dyslipidemia. (18, 19) As expected, in our sample, more women with obstructive CAD were on statins than women without CAD. But even when statin administration was controlled statistically, a significant difference remained. Third, we have a small sample with only seven women with obstructive CAD. Thus, our sample may not be representative of the population of women with and without obstructive CAD.

Emerging information in relation to CAD progression now focuses on TH2 (anti-inflammatory) cytokines and the imbalance between pro- and anti-inflammatory cytokines, rather than the level of pro-inflammatory cytokines alone. (17) Given the relatively young

age of this sample, the findings of unusual TH1/TH2 ratios calls out for further investigation. Having knowledge of the cytokine milieu may direct treatment to prevent the development or progression of CAD in young women. Further research conducted prior to and after statin initiation in women with multiple CAD risk factors may shed additional light on this phenomenon.

There are several limitations to this study. The pilot sample size is small, and participants had undergone clinically-indicated coronary angiography for suspected ischemic heart disease from one tertiary center in Los Angeles which may limit generalizability. Additionally, the number of cytokines detectable in the plasma of these women was limited. More sensitive cytokine assays are now available.(20) While statins are known to reduce inflammation (21), and our findings did not differ by statin use, we may have been underpowered to detect this. Finally, although samples were drawn with regard to menstrual cycling, the role of hormones is not considered in this analysis. Strengths of our study were the use of core labs for cytokine and coronary angiographic measurements.

Future Directions

These initial results suggest that premenopausal women with obstructive CAD have lower TH1:TH2/HR ratios compared to similar women without obstructive CAD. The findings suggest that young women with obstructive CAD may experience increased recruitment of reparative cells that secrete TH2 cytokines compared to women without CAD. Thus, cytokine balance may play a role in obstructive CAD in young women. Future work in larger samples of younger women with a wider array of circulating inflammatory marker profiles could be useful for development of risk assessment and preventive strategies.

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* $P < 0.05$

FIGURE 1. COMPARATIVE CYTOKINE PROFILES OF WOMEN WITH VS WITHOUT OBSTRUCTIVE CAD

The display of the median z-scores for each cytokine, where Th-1 Cytokines are presented clockwise from IL-1ra to TNF alpha, Th-2 Cytokine MCP-1, Hematopoietic/Regulatory cytokines (left side of diagram) IL-6-VEGf, IL-1ra = interleukin-1 receptor antagonist, RANTES = Regulated on Activation, Normal T Expressed and Secreted, TNFa = tumor necrosis factor alpha, MCP-1 = monocyte chemoattractant protein 1, IL-8 = interleukin 8

Table 1

Demographic and Clinical Characteristics by Obstructive CAD Status

Variable	Obstructive CAD (n = 7)	No Obstructive CAD (n = 17)	p*
Age (Medians [IQR])	44 [43, 47]	43 [41, 47]	0.63
White race (%)	43	76	0.17
BMI (Medians [IQR])	34.4 [24.2, 39.4]	29.0 [24.2, 38.1]	0.69
Education: HS or more (%)	86	100	0.29
Married (%)	71	65	>0.99
Income > 35K (%)	40	88	0.06
Hx Hypertension (%)	83	38	0.15
Hx Dyslipidemia (%)	71	53	0.65
Diabetes (%)	57	18	0.13
Family History of CAD (%)	71	41	0.37
Current smoker (%)	0	12	>0.99
DASI (Medians [IQR])	18.7 [17.0, 35.0]	30.7 [13.2, 50.7]	0.84

* p-values based on Kruskal-Wallis test (continuous) and Fisher exact test (frequencies) %= Per cent

DASI- Duke Activity Status Index

IQR= Interquartile Range

Table 2

Cytokine Ratios by CAD Status

Cytokine Ratio T1/T2:	No CAD (n=17)	CAD (n=7)	P (K-W)	P (GLM) unadjusted	P (GLM) adjusted
IL-1ra/MCP-1	10.6 ± 7.4	15.9 ± 18.6	0.72	0.67	0.69
IL-8/MCP-1	0.07 ± 0.07	0.02 ± 0.02	0.004	0.0005	0.0007
ENA-78/MCP-1	15.4 ± 13.7	4.0 ± 2.6	0.007	0.006	0.008
MIP-1b/MCP-1	0.31 ± 0.16	0.22 ± 0.14	0.24	0.28	0.29
RANTES/MCP-1	193 ± 87	120 ± 80	0.028	0.020	0.021
TNFα/MCP-1	0.06 ± 0.04	0.05 ± 0.03	0.59	0.24	0.25

Proinflammatory

IL-1ra = interleukin-1 receptor antagonist
RANTES = regulated on activation, normal T expressed and secreted
MIP-1 = macrophage inducible protein 1
TNFα = tumor necrosis factor alpha

Anti-inflammatory

MCP-1 = monocyte chemoattractant protein 1

Hematopoietic Regulatory

ENA-78 = epithelial-derived neutrophil-activating peptide 78
IL- = interleukin

* K-W = Kruskal-Wallis (nonparametric test)

* GLM = General Linear Models regression (parametric test)