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Temporal reliability of serum soluble and endogenous secretory receptors for advanced glycation end-products (sRAGE and esRAGE) in healthy women

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Abstract

Purpose—The soluble receptor for advanced glycation end products (sRAGE) and endogenous secretory RAGE (esRAGE) have been considered as biomarkers of several chronic diseases. However, the temporal reliability of their concentrations in the circulation is yet to be demonstrated. We evaluated whether a single measurement of serum sRAGE and esRAGE could serve as an estimate for usual serum levels in epidemiologic studies.

Methods—Serum sRAGE and esRAGE were measured using ELISAs in three yearly samples from 36 participants in the New York University Women's Health Study. The intraclass correlation coefficient (ICC) was used to evaluate temporal reliability.

Results—The intra- and inter-batch coefficients of variation were 3.0% and 14.8% for sRAGE and 6.5% and 34.7% for esRAGE, and decreased to 0.4% and 2.1% for sRAGE and 1.0% and 6.3% for esRAGE after log₂-transformation of the data. On the original scale, the ICCs of a single measurement of serum sRAGE and esRAGE were 0.89 (95% CI: 0.82, 0.94) and 0.87 (95% CI: 0.79, 0.93), respectively, and were similar using log₂-transformed data.

Conclusion—Our results indicate that a single measurement of serum sRAGE and esRAGE is a sufficiently reliable measure of their usual levels that can be used in epidemiologic studies.

Keywords

sRAGE; esRAGE; Biomarker; Reliability; ELISA; Epidemiology

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval The study was approved by the Institutional Review Board of New York University School of Medicine.

Informed consent Written informed consent was obtained from all the subjects at the time of enrolment.

Introduction

Advanced glycation end products (AGEs) and their receptor (RAGE) are implicated in a number of chronic diseases, including inflammation and cancer (1). AGEs are a group of irreversible adducts created through non-enzymatic reactions between reducing sugars (e.g., glucose and fructose) and free amino groups on proteins, lipids, and nucleic acids. AGEs form endogenously during normal metabolism and exogenously from foods processed at high temperatures and from tobacco smoke. AGEs accumulate slowly in human tissues and organs during aging and more rapidly in conditions associated with hyperglycemia and enhanced oxidative stress (2). AGEs elicit biological function through the receptor for AGEs (RAGE). The interaction between AGEs and RAGE activates an array of signaling pathways that are involved in inflammation and tumorigenesis (1). In addition, it has been reported that RAGE is also a receptor for non-AGE ligands.

Soluble forms of RAGE (sRAGE), including endogenous secretory RAGE (esRAGE) (3) and another C-terminally truncated form of RAGE that has been enzymatically cleaved from the cell surface (4), are detectable in the circulation of humans and are postulated to competitively bind with RAGE ligands, thereby potentially neutralizing circulating AGEs and other ligands and protecting against RAGE-mediated effects (5). Levels of circulating sRAGE and esRAGE have been inversely associated with cardiometabolic diseases (6, 7) in epidemiologic studies. Recently, several prospective studies have also assessed circulating levels of sRAGE and esRAGE in relation to risk of colorectal cancer, liver cancer, or pancreatic cancer (8–11).

In most prospective epidemiologic studies, only one blood sample is collected from each participant. If the within-subject variability in biomarker concentration is large relative to the between-subject variability, estimates of the association between the long-term average biomarker concentration, which is usually the exposure of interest for chronic diseases, and disease risk will be biased in studies using biomarker concentrations measured at a single point in time (12). Biomarker measurements in repeat samples collected at several time points from the same subjects can be used to estimate the temporal reliability of the biomarker, and thus the degree of measurement error in studies using a single sample. Such information can then be used in the planning, as well as in the interpretation of the results, of biomarker-outcome association studies. To date, no study has assessed the long-term reliability of serum sRAGE and esRAGE.

We conducted a study to evaluate the temporal reliability of a single measurement of serum levels of sRAGE and esRAGE using three yearly samples from 36 healthy women selected from the New York University Women's Health Study (NYUWHS). We also assessed laboratory variability of the measurements.

Materials and methods

New York University Women's Health Study

Details of the NYUWHS have been presented elsewhere (13). Briefly, a total of 14,274 women, aged 34–65 years, were enrolled between 1985 and 1991 at a breast cancer

screening center in New York City. Demographic, lifestyle, dietary, and medical information was collected using a self-administered questionnaire. Non-fasting blood was collected at enrollment and at annual repeat screening visits. Two or more yearly blood samples are available for 52% of the participants. Preparation and storage of serum samples were previously described (14).

Study design

We randomly selected 16 premenopausal women and 16 postmenopausal women from the NYUWHS who: (1) donated blood annually three or more times; (2) had not been diagnosed with diabetes, CVD or cancer at latest follow-up; and (3) had not been selected as controls in any case-control studies nested within the cohort. Serum samples donated at the first, second and third visits by these thirty-six women were used for this study.

Each sample was labeled with a unique identification number and the laboratory personnel were blinded to the identity of the participants and which samples were from the same participant. All three samples from a woman were run on the same batch. Twelve masked quality control replicates from a common pool were included randomly in the three batches (4 replicates/batch) to assess laboratory variability.

sRAGE and esRAGE measurement

Total sRAGE was measured by an enzyme-linked immunosorbent assay (ELISA) (Quantikine Human RAGE Immunoassay; R&D Systems, Minneapolis, MN). This assay measures all soluble forms of RAGE including esRAGE; however, it does not give values for esRAGE separately. The limit of detection (LOD) for the assay was 78 pg/ml. sRAGE was detected in 100% of the samples. Serum esRAGE was specifically measured using an ELISA (B-Bridge International, Mountain View, CA) with a LOD of 50 pg/ml. esRAGE was below the LOD in all three samples from two women and in two out of three samples from another woman. We imputed the esRAGE concentrations in these samples as the LOD divided by the square root of 2. Measurements were all performed in duplicate and the mean of the duplicate measurements was used in the statistical analysis.

Statistical analyses

We examined the laboratory variability using the intra- and inter-batch coefficients of variation (CVs). To assess reliability, we estimated the intraclass correlation coefficients (ICCs) and their 95% confidence intervals (CIs) (15) using PROC GLM in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Computation of the CVs and ICCs were based on both the raw and the log₂-transformed data. We used linear regression models to examine the associations of the serum levels of sRAGE and esRAGE in the first-year samples with age (years), body mass index (BMI, kg/m²), race, smoking status, education, and the length of sample storage (years) at the first blood donation. We estimated regression coefficients and their 95% confidence intervals (CIs) for the difference in serum levels of sRAGE and esRAGE in relation to per 1-year increase in age or length of sample storage, per 1-kg/m² increase in BMI, and comparing non-white to white, ever smokers to never smokers, and women with higher education level (college and higher) to those with lower education level (less than college).

Results

The mean age of the study participants at enrollment was 48.5 years, and the mean BMI was 24.8 kg/m². Seventy percent of the study participants were white, 51.4% were never smokers, and 48.3% had higher education (college and higher). Serum samples were stored for an average of 31.0 years.

Table 1 shows the descriptive statistics of annual and average levels of serum sRAGE and esRAGE. The ranges and means of serum sRAGE did not differ greatly by visit number, with a range of 499.8–2573.4 pg/ml for the average of the three yearly measurements. The 3-year average of esRAGE concentration ranged from 35.4 to 724.7 pg/ml, with a mean of 279.3 pg/ml (SD 192.0 pg/ml) for the first-year samples.

Table 2 displays the intra- and inter-batch CVs and the ICCs for the raw and log₂-transformed data. On the original scale, the intra- and inter-batch CVs were 3.0% and 14.8% for sRAGE and 6.5% and 34.7% for esRAGE. The CVs decreased appreciably after log₂-transformation of the data, with intra- and inter-batch CVs of 0.4% and 2.1% for sRAGE and 1.0% and 6.3% for esRAGE.

The ICCs for a single measurement of sRAGE and esRAGE were 0.89 (95% CI: 0.82, 0.94) and 0.87 (95% CI: 0.79, 0.93), respectively, on the original scale, and did not change materially after log₂-transformation of the data. There was no significant association of the serum levels of sRAGE and esRAGE with age, BMI, race, smoking status, educational attainment of the participants, or length of sample storage (Supplemental Table 1); however, serum levels of sRAGE tended to be lower in non-whites comparing with whites ($P=0.19$).

Discussion

For a biomarker, an ICC 0.75 indicates excellent reliability, 0.40 to 0.75 indicates fair to good reliability, and <0.40 indicates poor reliability. In our study that included three annual samples from thirty-six women, we observed excellent reliability for serum sRAGE and esRAGE, indicating that a single measurement is sufficient to rank women according to usual levels over a three-year period in our study population. The ICCs of sRAGE (0.89) and esRAGE (0.87) are substantially higher than those observed for serum cholesterol (ICC = 0.65), blood pressure (ICC = 0.60–0.64), blood glucose (ICC = 0.52), pulse (ICC = 0.49, and plasma estradiol in postmenopausal women (ICC = 0.68) over several years, all of which are exposures considered to be reasonably well-measured and have been repeatedly shown to be predictors of disease in epidemiologic studies (16).

A previous study evaluated variability of sRAGE in plasma samples collected three-years apart from 179 men and women and reported an ICC of 0.74 (17). Though this ICC was somewhat lower than what we observed in our study, results of these two studies support that circulating levels of sRAGE, measured either in serum or plasma, are quite stable over a long period of time and that a single measurement can be used to assess whether sRAGE is associated with the risk of chronic diseases.

Serum levels of sRAGE measured in this reliability study ranged from 499.8 to 2573.4 pg/ml with a mean of 1396 pg/ml, which are values similar in magnitude to those reported in previous studies of nondiabetic populations (7, 18, 19). Interestingly, we found that serum levels of sRAGE were lower in non-whites than whites, consistent with a number of previous studies showing significantly lower levels of sRAGE in non-Hispanic blacks and Hispanics in comparison to whites (6, 20–22). sRAGE levels in serum/plasma have been shown to be very stable under various adverse preanalytical conditions including delayed centrifugation, high storage temperature before and after centrifugation, and repeated freeze-thaw cycles (23, 24). Although the samples used in our study were frozen for over 30 years, levels of serum sRAGE were similar to the values reported in the literature using samples stored for a shorter time, suggesting that there is no degradation with long time in storage, at least at -80°C .

This is the first study to assess the reliability of serum esRAGE. We found that esRAGE levels were ~ 5-fold lower than total sRAGE levels, consistent with prior studies (25, 26). The sRAGE ELISA detects several components of total sRAGE proteins, including esRAGE, the cleaved RAGE, and other splice variants of RAGE. There are several reasons why measuring esRAGE in addition to total sRAGE may be of interest: (1) it was suggested that esRAGE is the only splice variant, capable of capturing AGE ligands (3); (2) the cleaved RAGE is part of a regulatory process and may reflect ongoing inflammation (4), in which case one would expect higher levels of sRAGE to be associated with higher disease risk; however as mentioned, total sRAGE levels have been inversely associated with a number of chronic diseases (6, 7); (3) the proportion of diverse components of sRAGE could differ among individuals; and (4) inconsistent findings have been observed for the associations of total sRAGE and esRAGE with disease (26). The high reliability of a single measurement of esRAGE reported in the present study supports future investigation of its use as a potential biomarker in epidemiologic studies.

Several studies have found an inverse relationship of circulating levels of sRAGE with the risk of colorectal cancer, pancreatic cancer, and liver cancer (8–10), while no association was observed for esRAGE and the risk of pancreatic cancer (11). Although the temporal reliability was not evaluated in these studies, our data suggest that measurement error specifically attributable to within-person variability in serum sRAGE and esRAGE levels over time is unlikely to substantially influence the results of these studies.

We observed a high inter-batch CV (34.7%) for esRAGE, which was due to one batch with relatively low replicate values. For many immunoassays, including ELISA, that rely on calibration experiments for biomarker quantification, inter-batch variability is common and may arise for a variety of reasons, such as true batch variation, experimental conditions including weather and light exposure, laboratory technician variability, and variability by lot number (27). Various approaches to calibrating data have been described to address the inter-batch variability (28). Studies examining the relation between serum esRAGE and disease risk should implement quality control procedures to preclude the possibility of null findings due to the batch effects in esRAGE measurements. Given the possible batch effects, nested case-control studies may be a better option than case-cohort studies. In nested case-

control studies, cases and their matched controls are analyzed in the same batch thus minimizing the impact of batch effects on case-control comparisons.

In conclusion, our results suggest that a single measurement of serum sRAGE and esRAGE can reliably assess a woman's average level over several years, supporting their use in epidemiologic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Descriptive statistics of annual and three-year average levels of serum sRAGE and esRAGE in the 36 women from the New York University Women's Health Study

	sRAGE (pg/ml)				esRAGE ^d (pg/ml)			
	First year	Second year	Third year	Three-year average	First year	Second year	Third year	Three-year average
Mean	1386.8	1417.0	1384.4	1396.1	279.3	274.7	266.0	273.3
SD	558	584.4	596.2	558.4	192.0	183.2	179.6	176.8
Median	1256.2	1371.7	1220.1	1257.2	238	217.5	200.5	221.2
Range	520.4–2833.1	511.9–2622.3	467.2–2631.8	499.8–2573.4	35.4–919	35.4–697	35.4–679	35.4–724.7
10th and 90th percentiles	706.3, 2423.2	682.4, 2299.4	710.1, 2435.5	754.9, 2207.0	88, 490	72, 571	84, 577	95.3, 503.3

^aesRAGE concentrations below the LOD ($n = 8$) were imputed as the LOD/ $\sqrt{2}$.

Intra- and inter-batch CVs (%) and ICCs (95% CIs) of serum sRAGE and esRAGE measurements in the 36 women from the New York University Women's Health Study^a

Table 2

	sRAGE (pg/ml)		esRAGE ^b (pg/ml)	
	Original scale	Log ₂ scale	Original scale	Log ₂ scale
Intra-batch CV (%)	3.0	0.4	6.5	1.0
Inter-batch CV (%)	14.8	2.1	34.7	6.3
ICC (95% CI)	0.89 (0.82–0.94)	0.91 (0.84–0.95)	0.87 (0.79–0.93)	0.92 (0.86–0.95)

^aThe mean (SD) levels of sRAGE and esRAGE measured in the QC samples were 1400.0 (96.3)-pg/ml and 307.9 (49.0)-pg/ml, respectively.

^besRAGE concentrations below the LOD (n = 8) were imputed as the $LOD/\sqrt{2}$